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TITLE: **PHARMACOLOGICAL PREVENTION AND REVERSION OF ERECTILE DYSFUNCTION AFTER RADICAL PROSTATECTOMY, BY MODULATION OF NITRIC OXIDE/cGMP PATHWAYS**

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**14. ABSTRACT**
During Year 2 an experimental article previously submitted was completed and extensively revised for its ulterior recent publication **(P-1)**, describing the time course of histological and functional changes affecting the penile corpora cavernosa after bilateral cavernosal nerve resection **(BCNR)** in the rat, as an experimental model for erectile dysfunction subsequent to radical prostatectomy for prostate cancer. This condition seriously affects the quality of life of a large fraction of male patients undergoing this operation and their partners. Therefore, studying the mechanism that triggers it and trying to develop a pharmacological therapy aiming to cure this type of erectile dysfunction, have considerable public health significance.
In addition, the completion of Aim 1 was considerably advanced by conducting treatments related to Experiment 3, to demonstrate the effects of a long-term administration of a dose of a PDE5 inhibitor (in this case sildenafil) at one half the dose previously reported by us **(P-2)** from the results of Experiment 2 during Year 1, as a preventive approach for CVOD and corporal fibrosis. A similar approach was based on the use of a nitric oxide donor, molsidomine, related to Experiment 2. Rat models related to BCNR were applied to establish the similarities and differences between acute (BCNR) and chronic (diabetes) damage to the penile corpora cavernosa, with the common goal of preventing CVOD and the underlying histopathology. All the pending animal treatments in the BCNR model that include lower doses and a different form of oral administration are ongoing, as well as a treatment for reversing the CVOD once established, for experiments 2 and 3. This will continue and be finalized during Year 3. The Year 2 results led to the publication of two additional papers as invited reviews **(P5-P6)**, making a total of six related publications and eight communications to scientific meetings since this grant started.

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## Introduction

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## Description of research accomplishments

### A. For Aim 1

**Experiment 1.** The paper that was submitted was finally accepted **(P-1)** after a revision that differs somewhat from the original manuscript, as stated below.

**Summary of paper 1 (P-1). Fibrosis and loss of smooth muscle in the corpora cavernosa precede corporal veno-occlusive dysfunction (CVOD) induced by experimental cavernosal nerve damage in the rat.**

This is an extensively revised version of the previously submitted paper, including new results, which is now published

**Introduction**. Corporal veno-occlusive dysfunction **(CVOD),** which usually is associated with a loss of smooth muscle cells **(SMC)** and an increase in fibrosis within the corpora cavernosa, can be experimentally induced by an injury to the cavernosal nerves. The corporal tissue then expresses inducible nitric oxide synthase **(iNOS),** presumably as an anti-fibrotic and SMC-protective response.

**Aims:** We studied the temporal relationship in the corpora between the expression of iNOS, other histological and biochemical changes, and the development of CVOD, after bilateral cavernosal nerve resection **(BCNR)** in the rat.

**Methods and main outcome measures**. Rats underwent either BCNR or a sham operation. Cavernosometry was performed 1, 3, 7, 15, 30, and 45 days (n=8/group) after surgery. Penile tissue sections were subjected to Masson trichrome staining for SMC and

collagen, and immunodetection for alpha smooth muscle actin **(ASMA)** as SMC marker, iNOS, neuronal NOS **(nNOS),** endothelial NOS **(eNOS),** proliferating cell nuclear antigen **(PCNA)** for cell proliferation, and TUNEL for apoptosis, followed by QIA. Quantitative western blot measured some of these markers in homogenates

**Results.** Following BCNR, CVOD was detectable 30 days later and it became more pronounced by 45 days. In contrast, the SMC/collagen ratio in the BCNR corpora was reduced at 7 days and bottomed at 30 and 45 days, due in part to the reduction of SMC, presumably caused by an increase in apoptosis peaking at 3 days but remaining high thereafter. Cell proliferation also peaked at 3 days but then decayed. nNOS was reduced early (3-7 days) and disappeared at 30 days, whereas eNOS was not affected. iNOS was induced at day 3, and steadily increased to high levels peaking at 30 days.

**Conclusions**. CVOD develops in the BCNR rat as a result of the early loss of corporal SMC by the neuropraxia-induced apoptosis, which the initial cell replication response cannot counteract, followed by fibrosis. The time course of iNOS induction supports the antifibrotic role of iNOS .

In our timetable of work, we had projected that during Year 2 we would carry out Experiment 2 within Aim 1. According to the SOW, this experiment was described as follows:

**Experiment 2. Effects of continuous oral administration of PDE5 inhibitors or NO generators on CVOD and the underlying corporal fibrosis, at a selected time after BCNR.** The aim is to investigate whether the BCNR-induced corporal fibrosis can be prevented at longer periods by either long-term continuous treatment with PDE5 inhibitors or a NO generators.

Rat groups will be injected as in Exp 1 and treated for 90 days with:

1) Vardenafil, in the drinking water
2) Molsidomine, in the drinking water
3) Vardenafil and molsidomine given together in the drinking water

In year 1 we had used another PDE5 inhibitor, sildenafil, instead of vardenafil, since we had completed the vardenafil study. Based on our papers **P-2-4** previously reported, we decided to modify the treatments above by continuing the study with sildenafil, because of its easier availability and solubility in the following way:

1) Sildenafil, in the drinking water at 10 mg/kg/day at half of the dose previously reported equivalent roughly to a 100 mg daily dose for men (considering surface differences for humans and rats)

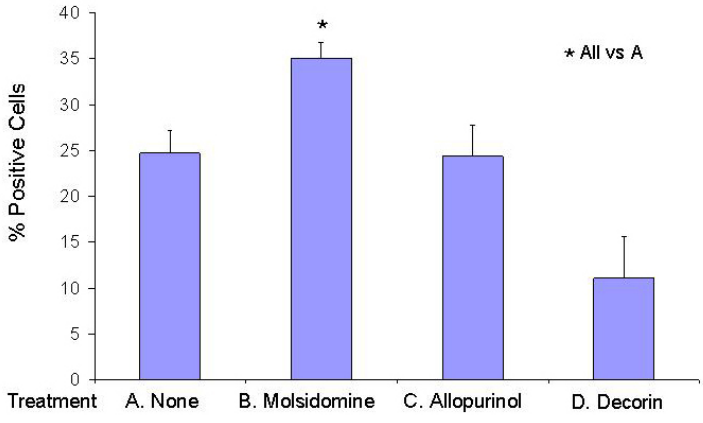


**Figure 1. Long-term molsidomine improves cell proliferation in the corpora cavernosa of a transgenic mouse where iNOS expression is genetically blocked.** Diabetic iNOS ko mice were treated for 45 days. Cell proliferation was measured by immunohistochemistry and QIA for PCNA

2) Molsidomine, given intraperitoneally daily (5mg/kg/day), 10-20 fold lower than maximal doses in the literature.

3) Sildenafil and molsidomine given together as in 1) and 2)

These treatments in the BCNR are ongoing (n=8/group) and the results will be reported in Year 1.

Before starting the BCNR treatments we proceeded to test these interventions in two other rodent models with corporal veno-occlusive

dysfunction **(CVOD)** and underlying fibrotic histopathology, similar to the conditions caused acutely by cavernosal nerve damage, but elicited by the chronic insult of hyperglycemia in diabetes.

The first model is the streptozotocin-induced type 1 diabetes in a transgenic mouse model where induction of inducible nitric oxide synthase **(iNOS)** is genetically blocked, the iNOS ko mouse. This is important since in paper **P-1** we observed a considerable iNOS induction in the BCNR rat model, and we then aimed to ascertain what the role of iNOS is in the putative prevention of penile fibrosis, in this case induced by diabetes. This protective role of iNOS in the penile corpora cavernosa of the diabetic iNOSko mouse **(Abstract A-2)** was confirmed, supporting the importance of iNOS spontaneous induction in defending this tissue from the fibrotic effects of endogenous or iatrogenic insults.

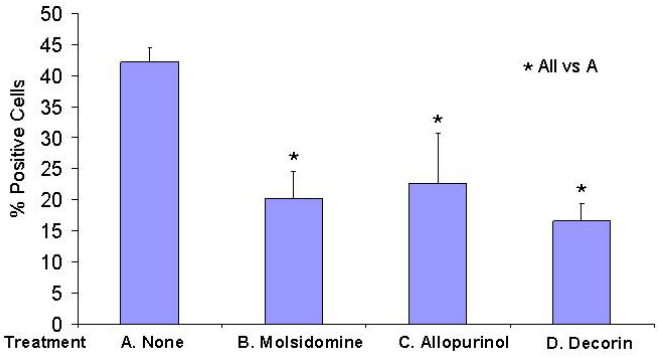


**Figure 2. Long-term olsidomine improves oxidative stress in the corpora cavernosa of a transgenic mouse where iNOS expression is genetically blocked.** Diabetic iNOS ko mice were treated for 45 days. Oxidative stress was measured by immunohistochemistry and QIA for XOR

These diabetic mice were treated then for 45 days with molsidomine, but in this case not with the other arms planned for Experiment 1 **(Abstract A-3** and Preliminary results). **Fig. 1** shows that molsidomine improved cell replication in the penile corpora cavernosa determined by proliferating cell nuclear antigen **(PCNA).** The same beneficial effects were observed on oxidative stress, as measured by a key enzyme, xanthine oxidoreductase **(XOR) (Fig. 2).** In contrast, allopurinol, an antioxidant targeting XOR activity, and decorin, that binds the profibrotic factor transforming growth factor β1 **(TGFβ1),** did not affect, or reduced, respectively, cell replication. Both agents did decrease oxidative stress, as expected **(Figs. 1 and 2).**

**Summary of Abstract 2 (A-2). Genetic blockade of inducible nitric oxide synthase (iNOS) intensifies the development of fibrosis in a mouse model of diabetes type 1**

**Objectives**: The spontaneous induction of iNOS has been proposed to act as an endogenous antifibrotic mechanism in penile tissues. The purpose of this study was to determine whether the genetic blockade of the iNOS expression in the iNOS knock out **(iNOSko)** mouse model intensifies fibrosis in the penile corpora cavernosa, and whether this is exacerbated by type 1 diabetes induced by streptozotocin and counteracted by insulin.

**Methods:** Eight weeks old male iNOSko and wild type (WT) mice were untreated or injected intraperitoneally with 150 mg/kg B.W streptozotocin (STZ), with or without insulin (0.05 IU/kg B.W daily). Insulin treatment started after the animals showed a glycemic value of 200 mg/dl or higher. After 7 weeks of treatment, control and treated mice were subjected to a fasting glucose tolerance with 1g bolus of glucose. At 8 weeks of treatment mice were sacrificed, body weights obtained, glycemia, glucosuria, nitrates and proteinuria were measured. Oxidative stress was measured by determining the GSH/GSSG in whole blood. Paraffin-embedded penile sections were subjected to Masson trichrome staining for smooth muscle cells **(SMC)**/collagen ratio, α-smooth muscle actin **(ASMA)** immunostaining as a marker of SMC content, TUNEL assay for apoptosis, PCNA immunohistochemistry as a marker of cell replication, and TGFβ1 as main profibrotic factor.

**Results** Body weights were reduced by STZ treatment. Glycemia, glucosuria, or proteinuria, were evident in most of the diabetic mice. Oxidative stress was exacerbated in

diabetic animals and reduced by insulin treatment. iNOS ko mice exhibited more oxidative stress than WT. The corporal SMC/collagen ratio and the SMC content were reduced as expected in the iNOS ko mice as compared to the WT mice. STZ-induced diabetes led to further reduction, that was completely prevented by insulin in the WT mice but only partially in the iNOS ko mice. STZ-induced diabetes increased corporal apoptosis and reduced corporal cell replication in the WT but not in the iNOS ko mice, and insulin normalized cell proliferation but not cell death.

**Conclusion**: The antifibrotic role of iNOS in the penile corpora cavernosa was confirmed in the iNOS ko/STZ mouse model. Insulin prevented these changes in the WT diabetic mice but could not correct the decreases induced by the iNOS genetic deletion. This supports the importance of iNOS spontaneous induction in protecting the penile corpora cavernosa from the fibrotic effects of hyperglycemia.

**Summary of abstract 3 (A-3). Antifibrotic and antioxidant therapies prevent the development of fibrosis in the penile corpora cavernosa associated with type 1 diabetes in the iNOS knock out mouse.**

**Objetives**: The development of penile fibrosis in diabetes is associated with an increase in oxidative stress and TGFβ1. As a consequence, a putative compensatory induction of inducible nitric oxide synthase **(iNOS)** causes a sustained output of nitric oxide **(NO)** which may act as an endogenous antifibrotic agent by quenching oxidative stress and reducing TGFβ1. The purpose of this work is to determine whether, in the absence of iNOS, a long-term treatment with an antioxidant or a TGFβ blocker prevents fibrosis of the corpora cavernosa in the diabetic iNOS knock out **(iNOSko)** mouse model.

**Methods:** Eight weeks old male iNOSko were made diabetic by injecting intraperitoneally (IP) with 150 mg/Kg B.W streptozotocin (n=8/group) and were either left untreated or treated with oral allopurinol (40 mg/kg/day, in the water) as an antioxidant, or decorin (50 ug, IP, twice), as an anti-TGFβ1 agent. At 8 weeks, blood was extracted for measuring glycemia and reactive oxygen species **(ROS)** by the GSH/GSSG ratio. Urine was collected for qualitative estimations of glucosuria, ketonuria, and proteinuria. Mice were then sacrificed, body weights obtained, and paraffin-embedded tissue sections from the skin-denuded penile shaft were subjected to Masson trichrome staining for the SMC/collagen ratio, and immunostaining for SMC content by $\alpha$-smmooth muscle actin (ASMA), and apoptosis by TUNEL, cell replication by PCNA, and for the pro-fibrotic factor TGFβ1, followed by quantitative image analysis.

**Results.** Body weights and hyperglycemia were not affected by allopurinol or decorin compared to the untreated diabetic iNOSko. Glucosuria, ketonuria, and proteinuria, were not affected by any treatment. In contrast, systemic ROS were reduced by allopurinol, but not affected by decorin. The corporal SMC/collagen ratio and the SMC content were increased by decorin, despite a decrease in cell replication, but allopurinol only increased SMC content.

**Conclusions.** In the absence of iNOS, long-term treatment with decorin reduced diabetes-related SMC loss and fibrosis in the corpora cavernosa probably by interfering with TGFβ1 signaling, but not with oxidative stress. In contrast, allopurinol reduced SMC loss by reducing oxidative stress probably through xanthine oxido reductase inhibition. This indicates that even in the absence of iNOS, a putative antifibrotic agent, inhibiting oxidative stress or TGFβ1 activity is effective to ameliorate corporal fibrosis in diabetes.

We also conducted additional preliminary studies with molsidomine and allopurinol, and also with sildenafil as PDE5 inhibitor, and L-NIL as iNOS inhibitor on a second rat model, prior to the ongoing experiment in the BCNR rat. This is the ZDF fa/fa rat, a model of type 2 diabetes,

where a very high hyperglycemia was expected to lead to CVOD and corpora cavernosal fibrosis, similar to the conditions we found in the BCNR rat. The results presented on **Abstract A-4** indicates that this was not the case, since these rats did not virtually undergo CVOD or corporal fibrosis, despite this was present, as in the BCNR model, in the less hyperglycemic but morbidly obese Zucker fa/fa rat. One of the factors that appeared to counteract CVOD in the ZDF fa/fa rat was a considerable iNOS and nNOS levels.

Treatment for 2 months with the inhibitor of iNOS activity L-NIL reduced also iNOS expression **(Fig. 3)**, but surprisingly the resistance of this strain to CVOD despite the long-term and intense hyperglycemia, was not overcome by iNOS inhibition, that in other conditions exacerbate fibrosis in the penis, so that the results for the moment are difficult to interpret. L-NIL did not affect cell replication in the corpora cavernosa as measured by PCNA, presumably in the smooth muscle **(Fig. 4).** Molsidomine did not affect iNOS expression and increased non-

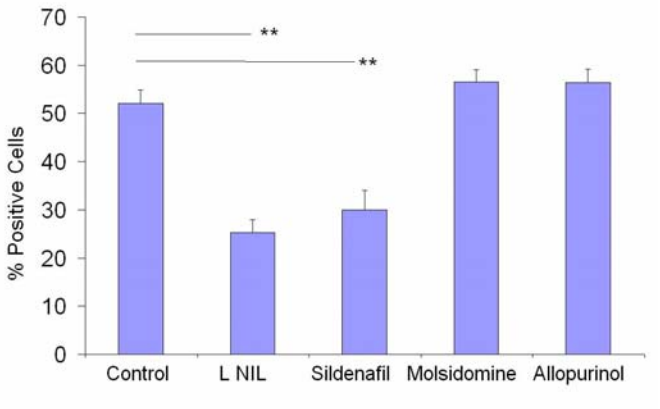


**Figure 3. Long-term sildenafil, but not molsidomine, reduce iNOS expression in the corpora cavernosa of a rat model of type 2 diabetes with considerable hyperglycemia but no obesity.** Diabetic iNOS ko mice were treated for 45 days. Cell proliferation was measured by immunohistochemistry and QIA for PCNA

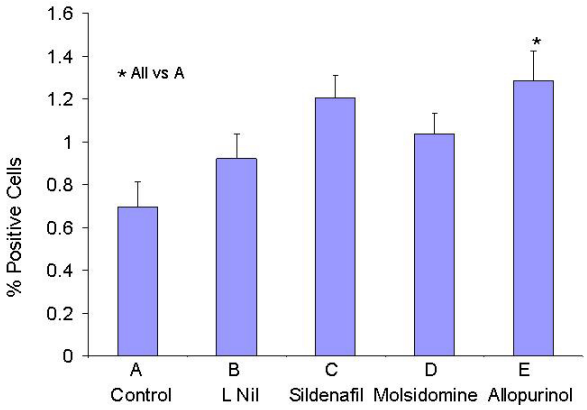


**Figure 4. Molsidomine and sildenafil improve cell proliferation in the corpora cavernosa of a transgenic mouse where iNOS expression is genetically blocked.** Diabetic iNOS ko mice were treated for 45 days. Cell proliferation was measured by immunohistochemistry and QIA for PCNA

significantly cell proliferation **(Figs. 3 and 4).** Sildenafil, also as expected**,** did increase cell replication, but reduced iNOS expression. Allopurinol also increased cell replication and did not affect iNOS expression.

These results will make simpler the interpretation of the new data with the BCNR, and have been integrated in the form of an invited review **(P-5; A-5-7)** on the comparison between the degree of CVOD and penile fibrosis in animal models of acute erectile dysfunction after cavernosal nerve damage during radical prostatectomy, and more chronic conditions such as diabetes. They were also used to extend the conclusions to another form of penile fibrosis, frequently associated with erectile dysfunction **(P-6, A-8).**

**Summary of abstract 4 (A-4). Severe hyperglycemia, renal damage, and low serum testosterone are insufficient in the absence of obesity to cause corporal veno-occlusive dysfunction (CVOD) in a rat model of type 2 diabetes**.

**Objective** We have shown that CVOD and a moderate corporal fibrosis occur in the obese Zucker fa/fa rat **(OZR)**, an animal model of morbid obesity, and type 2 diabetes with mild hyperglycemia. We also reported that both the OZR and a related strain with severe hyperglycemia, low serum testosterone, and no obesity, the Zucker diabetic fatty fa/fa rat

**(ZDFR),** develop a nephropathy with tubulointerstitial fibrosis. We determined whether these conditions are associated in the ZDFR with a more severe CVOD, corporal fibrosis, and iNOS induction as an antifibrotic mechanism, and whether these processes are exacerbated by iNOS inhibition.

**Methods** Male 6-month old ZDFR were maintained for 2 months (n=8/group) as follows: 1) no treatment; 2) L-NIL (inhibitor of iNOS activity) in the drinking water (1 mg/kg/day). Lean Zucker rats **(LZR)** were controls. Body weights and glycemia were determined weekly, and urine diabetes markers and serum T at completion. After dynamic infusion cavernosometry**,** penile shaft tissues were either frozen, or paraffin-embedded and sectioned for staining and quantitative image analysis for Masson trichrome (smooth muscle cell (SMC)/collagen ratio) and Oil red O (fat), and immuno-staining for iNOS, ASMA, iNOS, TUNEL (apoptosis), PCNA (cell replication), TGFβ1, and nNOS. Hydroxyproline in tissue hydrolyzates measured total collagen.

**Results** Body weights in the 8-month old diabetic ZDFR were similar to the LZR controls, and half of those in the OZR, but glycemia was 541 mg/dl (versus 244 mg/dl in the OZR) and serum T was <0.5 ng/ml in both strains, with glucosuria, and proteinuria. However, the CVOD seen in the OZR was absent in the ZDFR, and this strain had 2-fold higher SMC/collagen ratio, higher iNOS and nNOS content, and lower apoptosis than the OZR or LZR, with nearly normal ASMA content. The cavernosal SMC in the ZDFR was abundant and less compact (net-like), with no fatty infiltration seen in the OZR. No CVOD was induced by L-NIL, despite the 2-fold drop in the SMC/collagen ratio and iNOS expression.

**Conclusions** The absence of morbid obesity and fatty infiltration in the corpora, combined with a high SMC/collagen ratio, a peculiar SMC arrangement, and considerable iNOS and nNOS levels, oppose in the ZDFR the deleterious effects of severe hyperglycemia, renal dysfunction, and low serum testosterone on corporal tissue, and prevent CVOD. This resistance is not overcome by iNOS inhibition, that in other conditions exacerbate fibrosis in the penis.

**Summary of paper 6 (P-6) and abstracts 5-7 (A-5-A-7). Mechanisms of penile fibrosis**

**Introduction.** Penile fibrosis has been conceptually identified with the plaque tissue that develops in the tunica albuginea in Peyronie's disease **(PD)**, or with other localized processes induced in the corpora cavernosa by ischemic or traumatic events. Recently, it has recently been proposed that a diffuse, progressive, and milder intracorporal fibrosis, that affects also the media of the penile arteries, is responsible for vasculogenic erectile dysfunction **(ED)** associated with aging, smoking, diabetes, hypertension, and post-radical prostatectomy. These processes differ in etiology, time course, target cells, and treatment, but have many features in common.

**Aim.** To review the literature pertaining to fibrosis in the penis related to PD and ED, with emphasis on work carried out by the UCLA group.

**Methods**. PubMed search for pertinent publications mainly in the period 2001-2008

**Results.** This review focuses initially on PD and then deals with studies on ED in animal and cell culture models, discussing some of the pathophysiological similarities between tunical fibrosis in PD and corporal fibrosis in corporal veno-occlusive dysfunction **(CVOD),** and emerging common therapeutic strategies. The role of profibrotic factors, the resulting excessive deposit of collagen fibers and other extracellular matrix, the appearance of a synthetic cell phenotype in smooth muscle cells or the onset of a fibroblast-myofibroblast transition, and in the case of the corporal or penile arterial tissue the reduction of the smooth muscle cellular compartment, are discussed. This histopathology leads either to localized hardened plaques or nodules in penile tissues, or to the diffuse fibrosis causing impairment of tissue compliance that underlies CVOD and arteriogenic ED. The antifibrotic role of the sustained stimulation of the nitric oxide/cGMP pathway in the penis and its possible mechanism of action through the modulation of exogenous and endogenous stem cell differentiation is also briefly presented.

**Conclusions**. Fibrotic processes in penile tissues share a similar cellular and molecular pathophysiology, and common endogenous mechanisms of defense that have inspired novel pharmacological experimental approaches.

**Summary of paper 6 (P-6) and abstract 8 (A-8). Experimental models of Peyronie's disease. Implications for new therapies**

**Introduction.** Despite its high prevalence and impact on the quality of life of patients, and that it is an excellent model for the study of fibrotic processes, Peyronie's disease (PD) is an orphan disease in biomedical research. The development of animal and cell culture models has advanced substantially the understanding of its molecular and cellular pathology and the proposal of new therapies.

**Aim.** To review the literature pertaining to the use of these models for the study of PD.

**Methods.** PubMed search conducted from the first report of an animal model for PD.

**Results.** This model, based on the finding that TGFβ1 is overexpressed in the PD plaque, consists on the injection of TGFβ1 into the tunica albuginea of the rat. This leads to a PD-like plaque retaining many of the histological and biochemical features of human PD. Another rat model, based on the hypothesis that the PD plaque arises from trauma to the penis causing fibrinogen extravasation that initiates as fibrin a fibrotic response, consists on injection of fibrin into the tunica. The cell culture model is based on the demonstration that myofibroblasts are abundant in the human PD plaque.

**Conclusions.** These models have: a) clarified the role of microtrauma, myofibroblasts and oxidative stress in plaque development; b) demonstrated that this tissue is under sustained turnover by fibrotic and antifibrotic mechanisms; c) showed the interplay of collagenolytic and fibrinolytic systems and their inhibitors; d) detected an endogenous antifibrotic process consisting of the expression of inducible nitric oxide synthase that counteracts oxidative stress, collagen synthesis, and myofibroblast generation; e) characterized the antifibrotic effects of chronic treatment with PDE5 inhibitors; f) discovered the cytogenetic instability of PD cells and alterations in their gene expression; g) detected stem cells in the tunica albuginea with a potential role in fibrosis and ossification.

**Aim 2 Experiment 3. Comparison of treatment modalities involving PDE5 inhibitors.** The aim is to optimize the regimen, test longer-action agents, and determine whether treatment is effective when initiated after BCNR-induced functional and histological alterations are already present.

Rats will be subjected to BCNR (no sham operation) and divided in the following long-term treatment groups. This experiment (the last animal experiment for this grant) has already started, with slight modifications based on our previous data, as modified below:

<u>"Prevention" treatments</u>:

4) Continuous oral sildenafil, in the drinking water at 1/4 the previous dose, approximately 2.5 mg/kg/day

5) As in #4, plus molsidomine as in #3

6) Daily oral sildenafil, with retrolingual single daily instillation of 0.2 ml of a special formulation at 5 mg/ml **(9,16),** 1 mg/day, or approximately 2.5 mg/kg/day, thus at about ½ of the 50 mg equivalent tablet intake by men.

7) As in #6, plus molsidomine as in #3,

<u>"Reversion" treatment</u>:

8) Treatment selected from preceding groups, but initiated at 45 days post-BCNR and conducted for 45 days ("late treatment", for corrective therapy), using as a control the data previously obtained for a 90 days non-treated group.

9) Sham operation group

**Bulleted list of key research accomplishments**

We have demonstrated in a rat model of erectile dysfunction subsequent to cavernosal nerve damage during radical prostatectomy for prostate cancer, that:

- CVOD develops in the BCNR rat as a result of the early loss of corporal SMC by the neuropraxia-induced apoptosis, which the initial cell replication response cannot counteract, followed by fibrosis. The time course of iNOS induction supports the antifibrotic role of iNOS.

- The antifibrotic role of iNOS in the penile corpora cavernosa was confirmed in the iNOS ko/STZ mouse model of type 1 diabetes. This supports the importance of iNOS spontaneous induction in protecting the penile corpora cavernosa from the fibrotic effects of a chronic insult as hyperglycemia is, in a potential similar way as with cavernosal nerve damage.

- In the absence of iNOS in this transgenic mouse, long-term treatment with decorin reduced SMC loss and fibrosis in the corpora cavernosa probably by interfering with TGFβ1 signaling, but not with oxidative stress. In contrast, allopurinol reduced SMC loss by reducing oxidative stress probably through xanthine oxido reductase inhibition. This indicates that even in the absence of iNOS, a putative antifibrotic agent, inhibiting oxidative stress or TGFβ1 activity is effective to ameliorate corporal fibrosis.

- Treatment with molsidomine or sildenafil in the ZDFfa/fa rat, a model of type 2 diabetes improved corporal cell replication and oxidative stress but was irrelevant to the prevention of CVOD since these rats were found not to develop CVOD despite the considerable hyperglycemia.This resistance is not overcome by iNOS inhibition, that in other conditions exacerbate fibrosis in the penis.

- From the above, it is clear that ffibrotic processes in penile tissues share a similar cellular and molecular pathophysiology, as the underlying pathophysiology for erectile dysfunction, and common endogenous mechanisms of defense that have inspired novel pharmacological experimental approaches.

- These models of corpora cavernosa fibrosis have helped to clarify the role of endogenous antifibrotic process consisting of the expression of inducible nitric oxide synthase that counteracts oxidative stress, collagen synthesis, and myofibroblast generation and the antifibrotic effects of chronic treatment with PDE5 inhibitors in another fibrotic condition of the penis, Peyronie's disease.

**Reportable outcomes for Year 2**

Papers and abstracts follow a correlative numbering with those previously reported with the report for Year 1.

Papers acknowledging this grant PC061300 (W81XWH-07-1-0129) (see Appendix)

P-1: Ferrini MG, Kovanecz I, Sanchez S, Umeh C, Rajfer J, Gonzalez-Cadavid NF. Fibrosis and loss of smooth muscle in the corpora cavernosa precede corporal veno-occlusive dysfunction (CVOD) induced by experimental cavernosal nerve damage in the rat. J Sex Med. 2009 Feb;6(2):415-28. Epub 2008 Dec 2. PMID: 19138364 [PubMed - in process] Publication, previously listed in the Year 1 Report as paper 1, submitted.

P-5: Gonzalez-Cadavid NF. Mechanisms of penile fibrosis. J Sex Med. 2009 Mar;6 Suppl 3:353-62. PMID: 19267860 [PubMed - in process]

P-6. Gonzalez-Cadavid NF, Rajfer J. Experimental models of Peyronie's disease. Implications for new therapies. J Sex Med. 2009 Feb;6(2):303-13. Epub 2008 Dec 2. PMID: 19138365 [PubMed - in process]

Note: because of a confusion with the letters PR (which were incorrectly taken as abbreviating the terms "prostate research") there was an unfortunate confusion in the acknowledgement of papers 2 and 3 with another DOD grant: PR064756 (W81XWH-07-01-0181). This error was noticed during the preparation of this report. A request for an Erratum will be submitted to the Journal of Sexual Medicine, by substituting grant PC061300 for PR064756, in paper 2, and adding PC061300 to paper 3.

Abstracts and presentations related to this grant (penile fibrosis and CVOD)

A-2. Ferrini MG, J Moon J, Rivera S, Rajfer J, Gonzalez-Cadavid NF (2009) Genetic Blockade of inducible nitric oxide synthase (iNOS) Intensifies the Development of Fibrosis in a Mouse Model of Diabetes Type 1 Amer Urol Assoc (AUA) Ann Meet, Chicago, IL. Podium Presentation

A-3. Ferrini MG, Moon J, Rivera S, Vernet D, Rajfer J, Gonzalez-Cadavid, NF (2009) Antifibrotic and antioxidant therapies prevent the development of fibrosis in the penile corpora cavernosa associated with type 1 diabetes in the iNOS knock out mouse. Amer Urol Assoc (AUA) Ann Meet, Chicago, IL. Podium Presentation

A-4. Kovanecz I, Nolazco G, Ferrini MG, Rivera S, Vernet D, Rajfer J, Gonzalez-Cadavid NF (2009) Severe hyperglycemia, renal damage, and low serum testosterone are insufficient in the absence of obesity to cause corporal veno-occlusive dysfunction (CVOD) in a rat model of type 2 diabetes. Amer Urol Assoc (AUA) Ann Meet, Chicago, IL. Podium Presentation

A-5. Gonzalez-Cadavid NF (2008) Mechanisms of penile fibrosis. American Urological Association (AUA) Course, 08/20, Baltimore, MD. Invited speaker.

A-6. Gonzalez-Cadavid NF. (2009) Novel insights into the pathophysiology and therapy of erectile dysfunction in type 2 diabetes rat models in the context of reno-vascular disease. NIH Meeting for Urological Complications of Diabetes, 03/11, Baltimore, MD. Invited speaker

A-7. Gonzalez-Cadavid NF (2009) Role of fibrosis and inflammation in diabetes-related urological disorders. Cedars Sinai Meeting on Fibrosis and Inflammation Research, 03/19, 2009, Los Angeles, CA. Invited speaker

A-8. Gonzalez-Cadavid NF (2008) Advances in the understanding of Peyronie’s disease at the bench level. Sexual Medicine Society of North America (SMSNA) Ann Meet, 05/18. Orlando, FA. Invited speaker.

New applications for funding derived from preliminary results of this grant (penile fibrosis and CVOD)

The following grant applications have been submitted by investigators in this DOD grant using in part results obtained during year 1 of this grant.

1. Funded. PIs: Gonzalez-Cadavid NF, Rivera S (2008) Effects of Nitric Oxide/cGMP Modulators on Fibrosis of the Penile Corpora Cavernosa and Vasculogenic Erectile Dysfunction caused by Cavernosal Nerve Damage in a Rat Model. GCRC Medical Student Research Program. 11/08-10/09. No overlapping

2. Pending. PI: Gonzalez-Cadavid NF (2009). Modulation of human iPS differentiation in radical prostatectomy-related erectile dysfunction in rat models. NIH Recovery Challenge Grants. 09/09-08/11. No overlapping

3. Pending. PI: Gonzalez-Cadavid NF (2009). Erectile Dysfunction and Nitric Oxide Synthase in Aging. RO1 DK53069-07 (resubmission). 11/09-10/14. No overlapping

4. Pending. PI: Rivera, S, Mentor: Gonzalez-Cadavid NF (2009) Nitric oxide/cgmp modulation of stem cell differentiation for the treatment of vasculogenic erectile dysfunction caused by cavernosal nerve damage in rodent models. Sexual Medicine Society of North America (SMSNA) research grants. 07/09-06/10. No overlapping

Four other grant applications unrelated to the DOD project are pending.

The previously submitted grants for the NIH O'Brien Program on Erectile Dysfunction (see Report Year 1) were scored, including the program itself, but not funded

D. Appointments

In part because of the successful outcomes of this grant during the first year, the new principal investigator responsible for the LABioMed site during Year 2, Dr. Istvan Kovanecz, has been appointed as Assistant Professor in the Research Career Series at the UCLA Department of Urology.

## Conclusions

Cavernosal nerve damage, resembling the one caused by radical prostatectomy for prostate cancer, causes loss of smooth muscle cells and excessive collagen deposition in the penile corpora cavernosa. This is responsible for the impaired corporal compliance leading to corporal veno-occlusive dysfunction (CVOD), the prevalent form of vasculogenic erectile dysfunction that develops in many of these patients**.** The spontaneous induction of iNOS, leading to sustained higher levels of nitric oxide and cGMP, is an endogenous antifibrotic mechanism in the corpora cavernosa of this rat model that aims to counteract the pathophysiology of CVOD**.** Long-term continuous administration of PDE5 inhibitors immediately after cavernosal nerve damage, maintaining moderately high levels of cGMP, prevents CVOD and the underlying histopathology of the corpora cavernosa. This may soon be translated into the clinic, once the appropriate dosing is established, as a treatment to prevent or counteract erectile dysfunction after radical prostatectomy.

CVOD develops in the BCNR rat as a result of the early loss of corporal SMC by the neuropraxia-induced apoptosis, which the initial cell replication response cannot counteract, followed by fibrosis. The time course of iNOS induction supports the antifibrotic role of iNOS, Long-term tadalafil prevented CVOD and the underlying corporal fibrosis through a cGMP-related mechanism, independent of iNOS induction. This clarifies in the rat the sequence of

histological damages that lead to CVOD after radical prostatectomy, and suggests that the long-term continuous treatment with PDE5 inhibitors in men to prevent and/or counteract these conditions should be examined in controlled clinical trials.

## Summary of results

CVOD appeared late (30 days) in the BCNR rats as compared to the sham controls, and exacerbated at 45 days. This functional impairment was increased by continuous oral administration of the iNOS inhibitor L-NIL. In contrast, the SMC/collagen ratio in the BCNR corpora was reduced early (7 days) and bottomed at 30 and 45 days, due in part to the reduction of SMC, presumably caused by an increase in apoptosis peaking at 3 days but remaining high thereafter. Cell proliferation also peaked at 3 days but then normalized. nNOS was reduced early (3-7 days) and disappeared at 30 days, whereas eNOS was not affected. iNOS was induced at day 3, and steadily increased to high levels peaking at 30 days.

The moderate CVOD developing in the BCNR rat most likely results from the early loss of corporal SMC by the neuropraxia-induced apoptosis and the associated fibrosis, which the early cell replication response cannot counteract. The absence of eNOS decrease suggests that the endothelium is not affected to the same extent. The time course of iNOS induction and the exacerbation of CVOD and fibrosis by L-NIL support an antifibrotic role for iNOS.

PDE5 inhibitors, namely vardenafil, sildenafil, and tadalafil: 1) prevented the 30% decrease in the smooth muscle cell/collagen ratio, and the 3-4-fold increase in apoptosis and reduction in cell proliferation, and partially counteracted the increase in collagen, seen with both UCNR and BCNR; and 2) normalized the CVOD, measured by dynamic infusion cavernosometry, induced by both BCNR and UCNR. The long-term inhibition of iNOS activity exacerbated corporal fibrosis and CVOD in the BCNR rats, but sildenafil functional effects were not affected by L-NIL. These data suggest that the salutary effects of continuous long-term PDE5 inhibitors on erectile function post-cavernosal nerve resection involve their ability to prevent the alterations in corporal histology induced by cavernosal nerve damage, in a process apparently independent from endogenous iNOS induction.

Following BCNR, CVOD was detectable 30 days later and it became more pronounced by 45 days. In contrast, the SMC/collagen ratio in the BCNR corpora was reduced at 7 days and bottomed at 30 and 45 days, due in part to the reduction of SMC, presumably caused by an increase in apoptosis peaking at 3 days but remaining high thereafter. Cell proliferation also peaked at 3 days but then decayed. nNOS was reduced early (3-7 days) and disappeared at 30 days, whereas eNOS was not affected. iNOS was induced at day 3, and steadily increased to high levels peaking at 30 days. Tadalafil normalized erectile function, SMC and collagen content, and improved the increase in cell death.

## References

They are listed in the papers enclosed in the Appendix

## Appendices

They include:

1) The downloaded publications for papers 1-3

2) The biographical sketches of Drs. Gonzalez-Cadavid, Kovanecz, Vernet, Nolazco, and Rajfer

# Fibrosis and Loss of Smooth Muscle in the Corpora Cavernosa Precede Corporal Veno-Occlusive Dysfunction (CVOD) Induced by Experimental Cavernosal Nerve Damage in the Rat

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**ABSTRACT**

***Introduction.*** Corporal veno-occlusive dysfunction (CVOD), which usually is associated with a loss of smooth muscle cells (SMC) and an increase in fibrosis within the corpora cavernosa, can be induced by an injury to the cavernosal nerves. The corporal tissue expresses inducible nitric oxide synthase (iNOS), presumably as an antifibrotic and SMC-protective response.

***Aims.*** We studied the temporal relationship in the corpora between the expression of iNOS, other histological and biochemical changes, and the development of CVOD, after bilateral cavernosal nerve resection (BCNR) in the rat.

***Methods.*** Rats underwent either BCNR or sham operation. Cavernosometry was performed 1, 3, 7, 15, 30, and 45 days (N = 8/groups) after surgery. Penile tissue sections were subjected to Masson trichrome staining for SMC and collagen, and immunodetection for alpha smooth muscle actin, iNOS, neuronal NOS (nNOS), endothelial NOS (eNOS), proliferating cell nuclear antigen (PCNA), and terminal transferase dUTP nick end labeling (TUNEL). Quantitative western blot analysis was done in homogenates.

***Main Outcome Measures.*** Time course on the development of fibrosis and CVOD.

***Results.*** Following BCNR, CVOD was detectable 30 days later, and it became more pronounced by 45 days. In contrast, the SMC/collagen ratio in the BCNR corpora was reduced at 7 days and bottomed at 30 and 45 days, due in part to the reduction of SMC, presumably caused by an increase in apoptosis peaking at 3 days. PCNA also peaked at 3 days, but then decayed. nNOS was reduced early (3–7 days) and disappeared at 30 days, whereas eNOS was not affected. iNOS was induced at day 3, and steadily increased peaking at 30 days.

***Conclusions.*** CVOD develops in the BCNR rat as a result of the early loss of corporal SMC by the neuropraxia-induced apoptosis, which the initial cell replication response cannot counteract, followed by fibrosis. The time course of iNOS induction supports the antifibrotic role of iNOS. **Ferrini MG, Kovanecz I, Sanchez S, Umeh C, Rajfer J, and Gonzalez-Cadavid NF. Fibrosis and loss of smooth muscle in the corpora cavernosa precede corporal veno-occlusive dysfunction (CVOD) induced by experimental cavernosal nerve damage in the rat. J Sex Med 2009;6:415–428.**

***Key Words.*** Fibrosis; Erectile Dysfunction; Smooth Muscle; Nerve Sparing; Radical Prostatectomy; Penis; Nitric Oxide; cGMP; Collagen; Inducible Nitric Oxide Synthase; Apoptosis

## Introduction

Despite the use of nerve-sparing surgical techniques during radical pelvic surgery in men, the cavernosal nerves still appear to be somewhat susceptible to injury during the surgical procedure as evidenced by persistent and relatively high rates of erectile dysfunction in the immediate post operative period following such nerve sparing techniques [1–4]. The primary reason for this surgically induced impotence is corporal veno-occlusive dysfunction (CVOD) or

venous leakage [5–8] which becomes manifest whenever there is a decrease in the content of corporal smooth muscle cells (SMC) [9]. When this occurs, the remaining corporal smooth muscle mass is unable to achieve sufficient relaxation to attain the high intracorporeal pressures which are necessary for the passive occlusion of the veins that egress the corporal bodies as they traverse underneath and through the tunica albuginea of the penis.

We have previously demonstrated in the rat, in a model of cavernosal nerve resection, that CVOD is apparent at 45 days after the neural injury [10–13]. This functional impairment was associated with a decrease in the SMC mass and an increase in collagen content in the corporal tissue. In addition, we also observed a concomitant increase in the expression of the inducible nitric oxide synthase (iNOS) following bilateral cavernosal nerve resection (BCNR). Since we have shown in other experimental injury models that the upregulation of iNOS postinjury, presumably via the synthesis of NO, can act as an antifibrotic defense mechanism against the development of fibrosis, we then hypothesized that the iNOS may be acting in a similar manner on the corporal tissue in this BCNR model. The evidence to support this hypothesis comes from our finding that the long-term continuous oral administration of a PDE5 inhibitor, which is known to upregulate the action of nitric oxide, not only prevented both the BCNR-induced CVOD and the loss of the corporal SMC mass [10–12] normally seen following this type of injury, but there was the unexpected finding that the PDE5 inhibitors also enhanced replication of the corporal SMC themselves.

However, even though it has been well established that CVOD develops after BCNR and that iNOS expression is increased in the corporal tissue, the temporal relationship between these processes have never been fully elucidated. The aim of this study was to determine: (i) whether the development of the histological and biochemical changes that occur after BCNR precedes the onset of the CVOD; and (ii) when and how long does iNOS induction occur following such a neural injury. These observations would help establish the time frame of when to initiate treatment with PDE5 inhibitors following cavernosal nerve damage in order to achieve the optimum antiapoptotic and antifibrotic effect of these drugs.

## Materials and Methods

### *Animal Treatments*

Five month-old male Fisher 344 rats (Harlan Sprague–Dawley, San Diego, CA) were randomly divided into sham operated and BCNR groups. Animals were sacrificed at 1, 3, 7, 15, 30, and 45 days after surgery (n = 8 each group). BCNR was performed as previously described [9–12]. Animals were operated under aseptic conditions and isoflurane anesthesia. In supine position, a midline incision was done, the pelvic cavity was opened, and the bladder and prostate were located. Under an operating microscope, the major pelvic ganglion and its inflow and outflow nerve fibers were identified after removing the fascia and fat on the dorsolateral lobe of the prostate. The main branch of the cavernosal nerve is the largest efferent nerve, which runs along the surface of the prostatic wall. Above the main branch, there are another four to six small efferent fibers which also run toward the membranous urethra, considered as ancillary branches of the CN. In order to recognize the main cavernosal nerve, stimulation with an electrode to induce penile erection was applied. In the sham-operated group, both cavernosal nerves were identified but not resected. In BCNR, the main cavernosal nerves and ancillary branches were resected by removing a 5-mm segment. This procedure mainly eliminates the nitrergic non adrenergic non cholinergic (NANC) stimulation to the corporal smooth muscle that elicits its relaxation during penile erection, while also interrupting some vasoconstrictor neurotransmission through coalescent adrenergic fibers in the cavernosal nerve. All animal experiments were approved by the Institutional Animal Care and Use Committee at our institution.

### *Dynamic Infusion Cavernosometry (DIC)*

Cavernosometry was performed as previously described [10–12,14]. Briefly, basal intracavernosal pressure (ICP) was recorded, and 0.1 mL papaverine (20 mg/mL) was administered through a cannula into the corpora cavernosa. The ICP during tumescence was recorded as "ICP after papaverine." Saline was then infused through another cannula, increasing infusion rate by 0.05 mL/min every 10 seconds, until the ICP reached 80 mm Hg ("maintenance rate"). The "drop rate" was determined by recording the fall in ICP within the next 1 minute after the infusion was stopped.

### Histochemistry and Immunohistochemistry

After cavernosometry, animals were sacrificed, and the skin-denuded penile shafts were fixed overnight in 10% buffered formalin, washed, and stored in alcohol (70%) at 4°C until processed for paraffin-embedded tissue sections (5 μm). Adjacent tissue sections were used for: (a) Masson trichrome staining for collagen (blue) and SMC (red); (b) immunodetection with: (i) monoclonal antibodies against α-smooth muscle actin (ASMA) as a SMC marker (Sigma kit, Sigma Diagnostics, St Louis, MO, USA) and proliferating cell nuclear antigen (PCNA) as marker of cell proliferation (Chemicon, Temecula, CA, USA); (ii) polyclonal antibody against iNOS [15] (Calbiochem, La Jolla, CA, USA); (iii) monoclonal antibody against eNOS [16] (Calbiochem); (iv) monoclonal antibody against nNOS [17] (Calbiochem). The specificity of the antibodies was validated by western blot.

Sections were then incubated with biotinylated antimouse IgG (ASMA PCNA, eNOS, nNOS) or biotinylated antirabbit IgG (iNOS), respectively, followed by avidin-biotinylated enzyme complex (Vector Labs, Temecula, CA, USA) and 3,3′diaminobenzidine (Sigma) (PCNA and iNOS), or with the ASMA Sigma kit (ASMA) and 3-amino-9-ethylcarbazole. TUNEL assay was performed as described [10–13] by applying the Apoptag peroxidase detection assay (Chemicon) with TdT enzyme and antidigoxigenin-conjugated peroxidase and 3,3′diaminobenzidine/H2O2. Sections were counterstained with hematoxylin. Negative controls in the immunohistochemical detections were done by replacing the first antibody with IgG isotype. The negative control for TUNEL was by substituting buffer for the TdT enzyme. Testicular tissue sections were used as positive control.

### Quantitative Image Analysis (QIA)

QIA was performed by computerized densitometry using the ImagePro 4.01 program (Media Cybernetics, Silver Spring, MD, USA), coupled to an Olympus BHS microscope (Olympus America, Inc., Melville, NY, USA) equipped with an Olympus digital camera [11–15]. For Masson staining, 40× magnification pictures of the penis comprising half of the corpora cavernosa were analyzed for SMC (stained in red) and collagen (stained in blue), and expressed as SMC/collagen ratio. For ASMA and iNOS staining, only the corpora cavernosa were analyzed in a computerized grid and expressed as % of positive area vs. total area of the corpora cavernosa. For PCNA and TUNEL determinations, the number of positive cells at 400× was counted, and results were expressed as a % of positive cells/total cells in the corpora cavernosa. In all cases, two fields at 40× (both sides of the corpora cavernosa) or eight fields at 400×, were analyzed per tissue section, with at least four matched sections per animal and eight animals per group.

### Western Blot Analysis

Penile tissue homogenates (100 mg tissue) were obtained in tissue protein extraction reagent (PIERCE, Rockford, IL, USA) and protease inhibitors (3 μM leupeptin, 1 μM pepstatin A, 1 mM phenyl methyl sulfonyl fluoride), and centrifuged at 10,000 g for 5 minutes. Supernatant proteins (30–50 μg) were subjected to western blot analyses [17–20] by 7–10% Tris-HCl polyacrylamide gel electrophoresis (Bio-Rad, Hercules, CA, USA) in running buffer (Tris/glycine/sodium dodecyl sulfate). Proteins were transferred overnight at 4°C to nitrocellulose membranes in transfer buffer (Tris/glycine/methanol), and the next day, the nonspecific binding was blocked by immersing the membranes into 5% nonfat dried milk, 0.1% (v/v) Tween 20 in phosphate buffered saline (PBS) for 1 hour at room temperature. After several washes with washing buffer (PBS Tween 0.1%), the membranes were incubated with the primary antibodies for 1 hour at room temperature. Monoclonal antibodies were as follows: (i) ASMA, as described above (1/1,000) (Calbiochem); (ii) glyceraldehide-3-phosphate dehydrogenase (GAPDH) (1/10,000) (Chemicon International); and (ii) PCNA (Chemicon International). The washed membranes were incubated for 1 hour at room temperature with 1/3,000 dilution (antimouse), followed by a secondary antibody linked to horseradish peroxidase. After several washes, the immunoreactive bands were visualized using the enhanced chemiluminescence plus western blotting chemiluminescence detection system (Amersham Biosciences, Piscataway, NJ, USA). The densitometric analyses of the bands were performed with Image J (NIH, Bethesda, MD, USA). A positive control was run throughout all gels for each antibody to standardize for variations in exposures and staining intensities. Negative controls were performed omitting the primary antibody. Band intensities were determined by densitometry and corrected by the respective intensities for a housekeeping protein, GAPDH, upon reprobing.

### *Statistical Analysis*

Values were expressed as mean ± standard error of the mean. The normality distribution of the data was established using the Wilk–Shapiro test. Multiple comparisons were analyzed by a two-factor (time and treatment) analysis of variance (two-way ANOVA), followed by post hoc comparisons with the Bonferroni test, according to the GraphPad Prism V 4.1 (GraphPad Software, Inc., La Jolla, CA USA). Differences were considered significant at $P < 0.05$.

## Results

### *Alterations in the SMC/Collagen Ratio in the Corpora Cavernosa Precede the Onset of CVOD Following BCNR*

DIC was performed at 1, 3, 7, 15, and 30 days after cavernosal nerve injury in order to determine when CVOD occurs post-BCNR. DIC values for the 45-day time period were taken from one of our previous articles with identical sets of BCNR- and sham-operated rats [11]. However, in all the subsequent figures for histological observations, the representative micrographies for 15 and 45 days are omitted to reduce space. Figure 1 (top) shows that the peak ICP following papaverine injection was not significantly affected by BCNR during the observed 30 days postinjury, although at 45 days after surgery, the value was significantly reduced. The drop rate, however, began to slowly increase by 7 days, but only became significant by 30 days and markedly progressed by 45-day postinjury.

Evaluation of the smooth muscle and collagen content within the corpora was then performed in cross-sections of the penile tissue harvested from the animals following performance of DIC. Figure 2 (top) shows that there does not appear to be any obvious visual changes in the Masson trichrome staining for collagen and SMC on representative micrographs in the sham groups throughout the experiment. However, a progressive intensification of the collagen deposition (stained in blue) and a reduction in the smooth muscle (stained in red) started to be visualized at day 7 after BCNR. When QIA was performed (Figure 2, bottom), an alteration in the SMC/collagen ratio is detected as early as day 3 postinjury, which becomes significantly severe by day 7 and remains so for the remainder of the study. The red staining of the SMC was easily differentiated from the red blood cells, which were not considered in the QIA determinations.

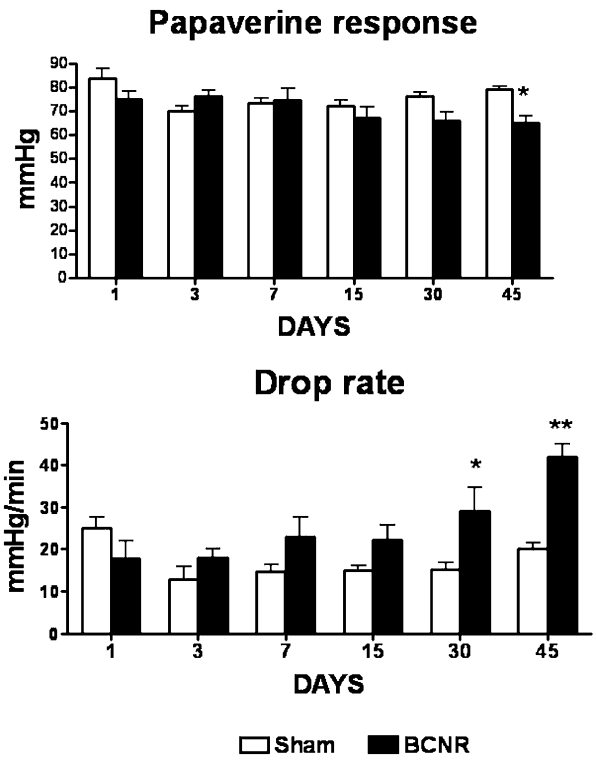


**Figure 1** Time course of effect of bilateral cavernosal nerve resection on the erectile function of the rat measured by pharmacological and infusion cavernosometry. Top: Response of the intracavernosal pressure to papaverine; Bottom: Response of the intracavernosal pressure to the interruption of saline infusion. *$P < 0.05$; **$P < 0.01$. SHAM = sham-operated animals; BCNR = animals subjected to nerve resection and killed at 1, 3, 7, 15, 30, and 45 days after surgery.

Picrus sirius red assays and observation under polarized microscope were done in adjacent sections to the ones used for Masson in order to discriminate the collagen III/I ratio. We have found that at the time points 7 and 30, there is an increase in collagen III/I ratio toward more production of collagen III, whereas at 45 days, the ratio is inverted to more collagen I than III (not shown). This difference could be due to the fact that the rate of collagen III synthesis is much faster than collagen I.

A second procedure to estimate SMC content based on the immunohistochemical determination of ASMA, an accepted marker of SMC in the corpora cavernosa, was also performed. Figure 3 (top) shows a considerable reduction with time in ASMA staining in the BCNR group as compared with the sham group as early as 3 days after BCNR that progressively worsens at 30 and 45 days. The respective reductions in

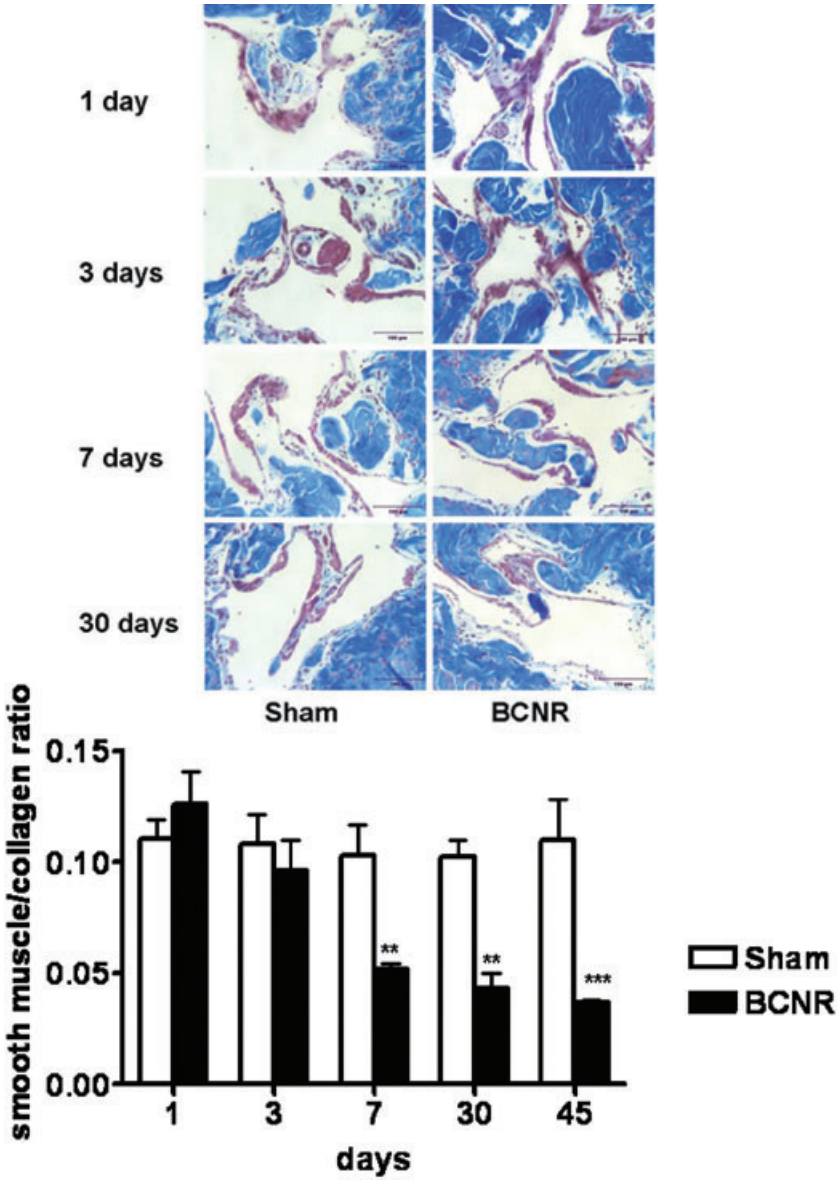


**Figure 2** Time course of the effect of bilateral cavernosal nerve resection on the smooth muscle/collagen ratio in the rat corpora cavernosa. Penile corpora cavernosa tissue sections from the rat groups presented on Figure 1 were stained with Masson trichrome. Top: representative pictures (200×, Bar = 100 μm). Bottom: quantitative image analysis. $^{**}P < 0.01$, $^{***}P < 0.001$. SHAM = sham-operated animals; BCNR = animals subjected to nerve resection and killed at 1, 3, 7, 30, and 45 days after surgery.

ASMA content determined by QIA (Figure 3A, bottom) were 40%, 76%, and 78% at 7, 30, and 45 days, respectively, postinjury. The expression of ASMA in the sham-operated group remained unchanged throughout the experiment (not shown). When western blot analysis of ASMA expression in homogenates of penile shaft tissue (Figure 3B, bottom) was performed, it paralleled the immunohistochemical measurements. Collectively, these results suggest that the histological changes induced by BCNR precede, as expected, the functional impairment of vasculogenic erectile response, and that the earliest event is SMC loss rather than collagen deposition. This is based on the fact that the reduction in ASMA + cells is rather considerable at a period (3 days) when the SMC/collagen ratio has only slightly decreased.

### *BCNR Results in a Decrease in nNOS, an Increase in iNOS and No Change in eNOS Content in the Corpora*

Since BCNR causes damage to the axons of the cavernosal nerve, Figure 4A confirms by immunohistochemistry with an antibody selective for nNOS a decrease in nNOS staining that is seen in cross-sections of the cavernosal nerve as early as 24 hours following the nerve injury. This antibody does not cross-react with eNOS and iNOS. Because the decrease in staining intensity was

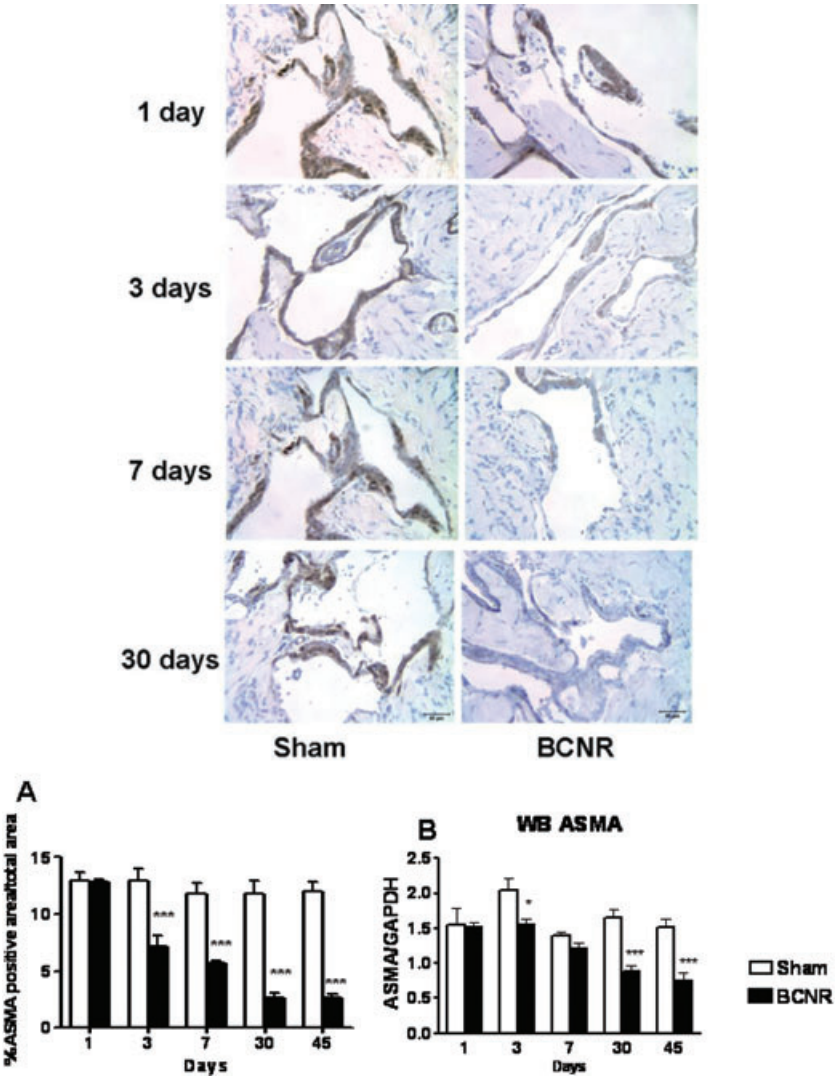


**Figure 3** Time course of the effect of bilateral cavernosal nerve resection on the smooth muscle cell content in the rat corpora cavernosa. Penile corpora cavernosa sections adjacent to those presented on Figure 2 were immunostained for α-smooth muscle actin (ASMA) as a smooth muscle cells marker. Top: 400×, Bar = 50 μm. A, Quantitative Image Analysis of Asma expression by immunohistochemistry. B, ASMA Expression by western blot analysis on penile homogenate extracts. $*P < 0.05$; $***P < 0.001$. Sham = sham-operated animals; BCNR = animals subjected to nerve resection killed at 1, 3, 7, 30, and 45 days after surgery.

so evident, no quantitative determination was deemed necessary to corroborate the visual inspection. In contrast, no changes were appreciable in the immunohistochemical detection of eNOS, which was constrained to the endothelium lining of the corpora cavernosa lacunar spaces or cisternae (Figure 4B). This was confirmed by QIA.

In an even more marked contrast to nNOS decrease, iNOS immunostaining in the corpora of the BCNR rats started to increase by 10-fold at day 3, and continued to remain high throughout the study period, while it stayed almost undetectable in the sham-operated animals at all time periods (Figure 5, top). The quantitative determination indicated that iNOS expression reached a peak at 30 days, when there was about a 50-fold increase over both the sham-operated and the preinjury values (bottom).

### *The Reduction in SMC Occurring After BCNR Is Due to an Early Peak of Apoptosis That Initially Is Compensated by Increased Cell Proliferation But Later on Predominates Over This Process*

TUNEL immunodetection assay revealed that by 1 day, and more so at 3 days following BCNR, there was a marked increase in apoptosis of cells in the corpora (not shown), and this was confirmed by QIA, which showed that the peak of apoptosis occurred at 3 days with a fivefold increase in the apoptotic index in the BCNR animals. This was followed by a gradual reduction, but still showing an over twofold higher apoptotic index at 45 days after BCNR (Figure 6, top).

When cell proliferation was measured by immunohistochemistry for PCNA, there was an intensification of cell proliferation at 1 and 3 dayspost-BCNR, but this level was subsequently

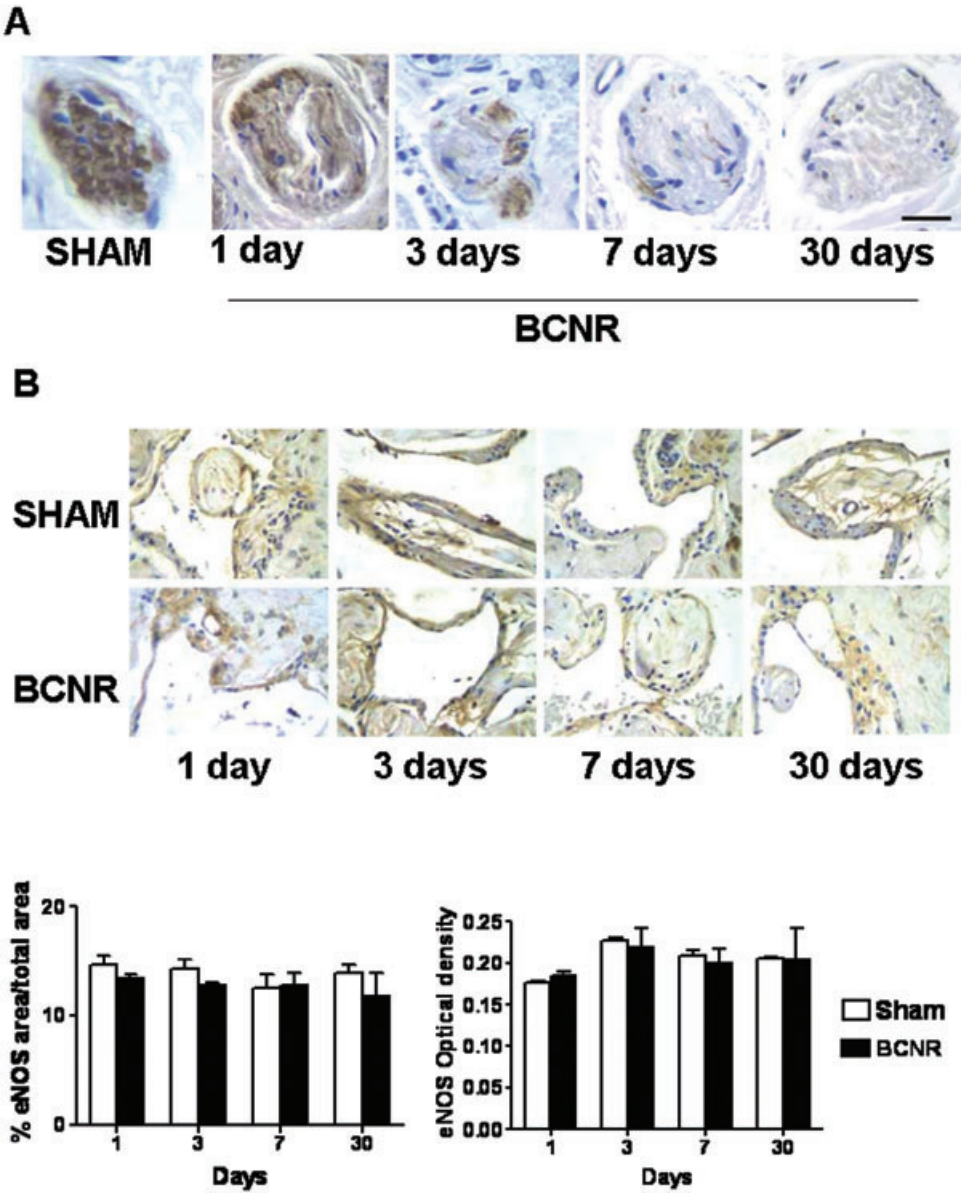


**Figure 4** Time course of neuronal NOS (nNOS) expression after nerve resection in cavernosal nerve terminals and of endothelial NOS (eNOS) in the corporal enothelium. (A) penile sections adjacent to those presented on Figure 2 were immunostained for nNOS. Magnification: 400×, bar = 50 μm. (B) sections adjacent to those presented on Figure 2 were immunostained with an eNOS antibody. The expression of eNOS is not altered by nerve resection. Top: 400×, bar = 50 μm. Bottom: quantitative image analysis. Sham = sham-operated animals; BCNR = animals subjected to nerve resection killed at 1, 3, 7, 30, and 45 days after surgery.

reduced by day 7 postinjury to basal levels (Figure 6, middle). QIA showed that, as in the case of apoptosis, the cell proliferation peak occurred at 3 days, with a similar fivefold increase in PCNA staining, which decreased thereafter. Interestingly, at 30 and 45 days post-BCNR, the PCNA values in the BCNR groups were lower than in the control sham-operated animals. Because of the initial stimulation of cell replication, the ratio between the proliferation and apoptotic indexes in the corpora (bottom) remains around a value of 1 until 7 days after BCNR, with no significant differences between BCNR and the sham-operated rats. However, at both 30 and 45 days, there is a considerable reduction in PCNA due to the predominance of cell death over cell proliferation. This agrees with the time course for SMC content in Figure 3.

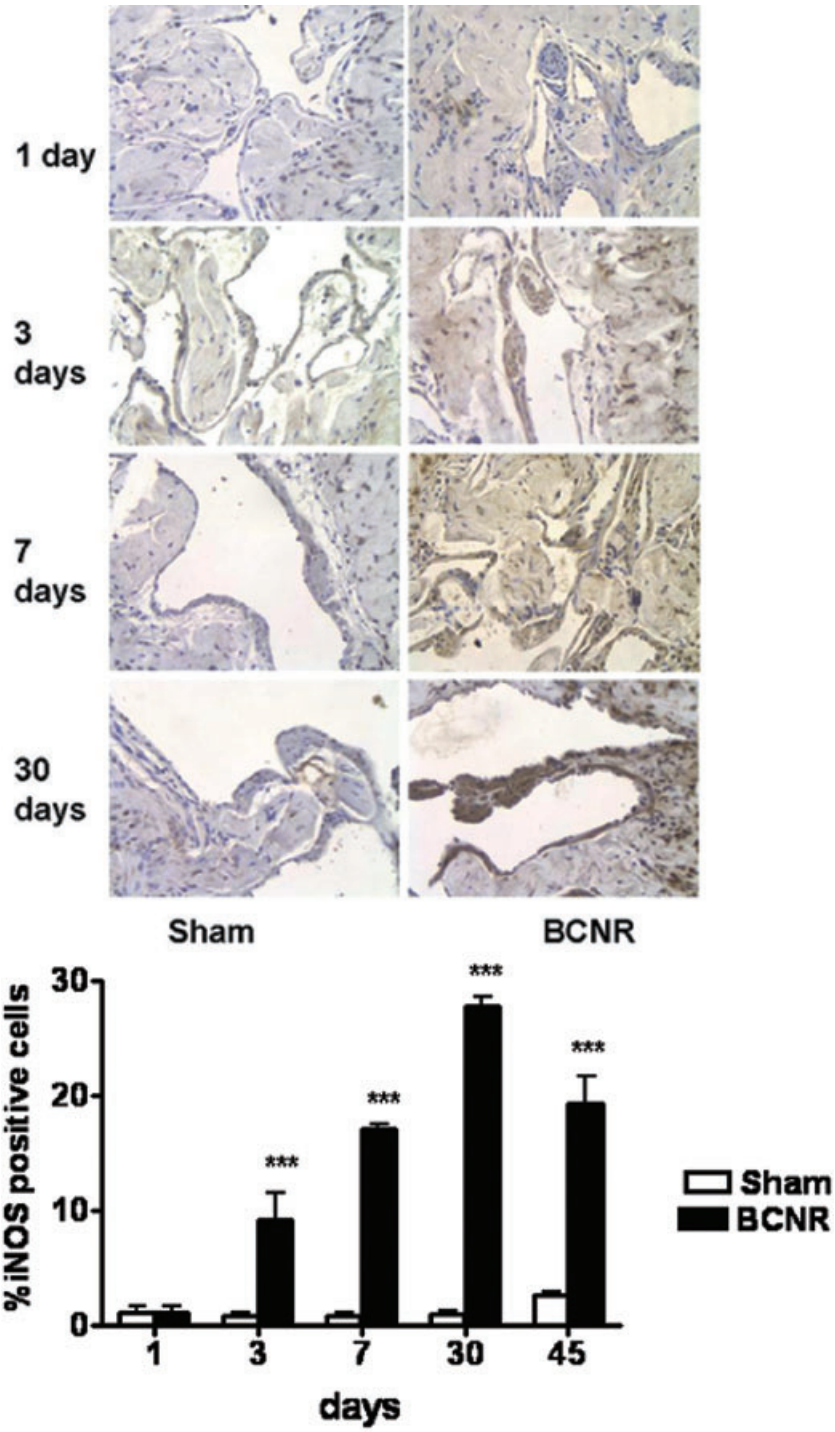


**Figure 5** Time course of the effect of bilateral cavernosal nerve resection on the expression of inducible nitric nitric oxide synthase (iNOS) in the penile corpora cavernosa. Penile corpora cavernosa sections adjacent to those presented in Figure 2 were subjected to immunodetection for iNOS. Top: representative pictures (200×). Bottom: quantitative image analysis. ***$P < 0.001$. Sham = sham-operated animals; BCNR = animals subjected to nerve resection killed at 1, 3, 7, 30, and 45 days after surgery.

The western blot analysis of PCNA expression in total penile shaft homogenates (Figure 7) confirmed the decrease in PCNA staining seen by immunohistochemistry in the tissue sections of the corpora cavernosa of BCNR rats (Figure 6, middle panel). However, the levels of PCNA in the homogenates of the penile shaft (Figure 7) were inconsistently high at the two earliest time periods, probably reflecting the presence of tunical and corpus spongiosum tissue (not considered in the analysis of the tissue sections of Figure 7).

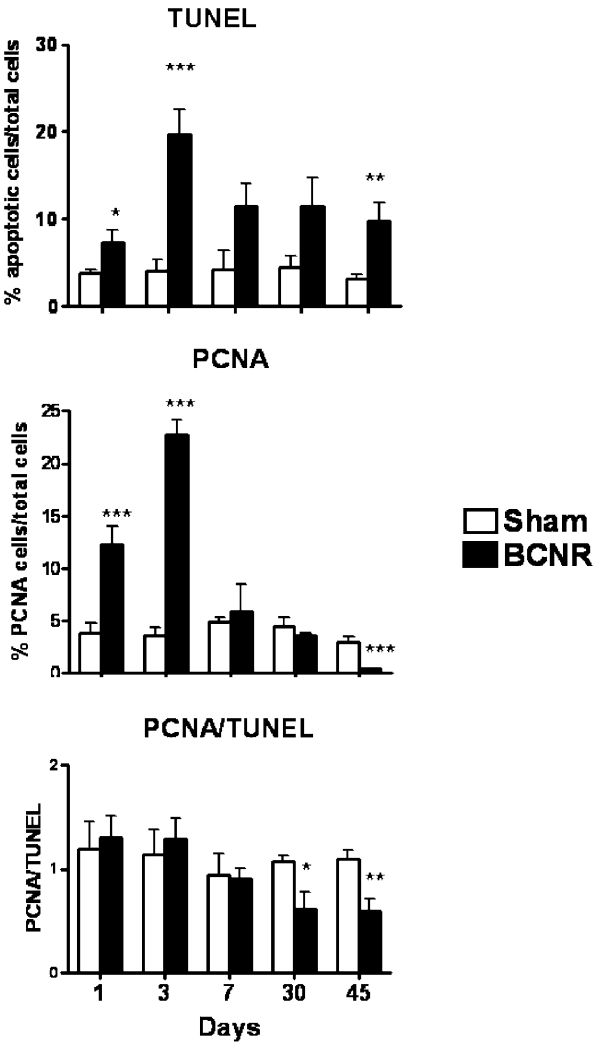


**Figure 6** Time course of the effect of bilateral cavernosal nerve resection on the cell turnover in the rat corpora cavernosa. Penile corpora cavernosal sections adjacent to those presented on the preceding figures were subjected to TUNEL and proliferating cell nuclear antigen (PCNA) staining. Top: quantitative image analysis (QIA) for TUNEL. Middle: QIA for PCNA. Bottom: the ratio between the total area occupied by cells undergoing cell replication (PCNA+) and the apoptotic index obtained above was established for each animal, and then used to calculate means ± SEM. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Sham = sham-operated animals; BCNR = animals subjected to nerve resection killed at 1, 3, 7, 30, and 45 days after surgery; Tunel = Terminal Transferase dUTP Nick End Labeling; SEM = standard error of the mean.

## Discussion

The current results clarify the sequential events that lead to the development of CVOD in the rat following cavernosal nerve damage. The assumption is that CVOD or venous leakage occurs because the SMC mass in the corpora is impacted in such a way that it cannot achieve sufficient relaxation to attain an intracorporeal pressure high enough that can compress the subtunical veins as they egress from the tunica albuginea of the penis. Normally, this is evident by a decrease in the SMC content, together with an increase in tissue fibrosis within the corpora.

The absolute amount of corpora smooth muscle that only drops significantly at day 30, but not at 7, approaching the value at day 45, appears to be more critical for corporal compliance and venous occlusion than the smooth collagen content ratio that falls down earlier. This interpretation would explain the fact that the significant increase in drop rate occurs at day 30 but not at 7 days, thus implying that a certain threshold in the corporal smooth muscle content combined with collagen deposition may be needed, below which the functional impairment would become evident.

The absence of a parallel significant decrease in the papaverine response at day 30 (despite the trend seen on Figure 1) may be due to the relatively high papaverine dosage (100 μL of 20 mg/mL solution, which is approximately 5 mg/kg. body weight) used in this study. This may be excessive to detect a marginal CVOD, based on the erectile response to the drug. However, we have recently conducted a papaverine dose/response titration curve during DIC in the rat, and we have found that 15 mg/mL of papaverine is an optimal concentration (or 3.8 mg/kg) for performing DIC, and this intracorporal dose will be used in the future.

Therefore, if the hypothesis of the decrease in the SMC content, together with an increase in tissue fibrosis within the corpora is correct, then apoptosis should occur first, followed by an observed decrease in the corporal smooth muscle content in combination with an increase in tissue fibrosis before CVOD becomes evident. Indeed, in our animal model of BCNR, the process of apoptosis is apparent 24 hours following the neural injury, an observation that has been previously reported by others [21–24]. What our data does show for the first time is that this apoptotic process peaks around 3 days following BCNR, and, although there is a slight decrease from this peak level seen after day 3, the level of apoptosis continues to remain elevated up to the end of the experiment, which was 45 days after BCNR.

The data confirm the observation of previous investigators [20–23] that programmed cell death is apparent as early as 1 day after the onset of

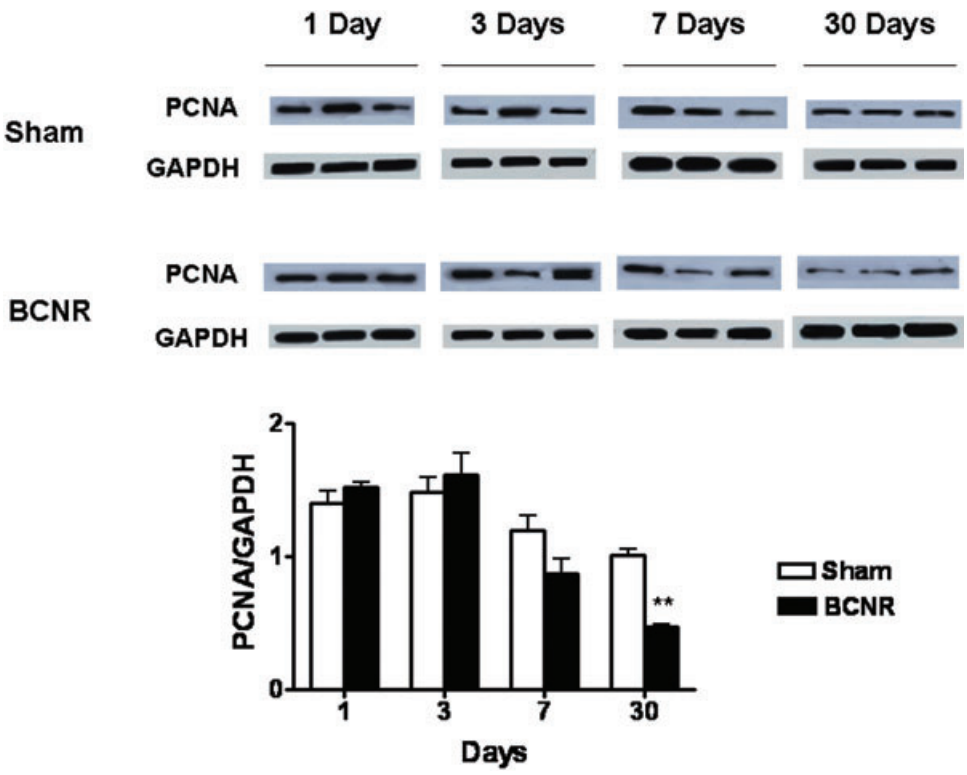


**Figure 7** Corroboration of the proliferating cell nuclear antigen (PCNA) immunostaining by western blot. Homogenates from corpora cavernosa tissue were subjected to western blot analysis with the same antibody used for Figure 6. $^{**}P < 0.01$. GAPDH = glyceraldehide-3-phosphate dehydrogenase. Sham = sham-operated animals; BCNR = animals subjected to nerve resection killed at 1, 3, 7, 30, and 45 days after surgery.

the neural injury. In addition, while the peak for these proapoptotic processes occurs by day 3 following the neural injury, there also appears to be a considerable increase in cell proliferation within the trabecular tissue around the cisternae, a finding that has not been previously reported, and one that may represent an attempt by the tissue itself to counteract apoptosis. Thereafter, cell proliferation, by drastically declining already at 7 days, becomes insufficient to counteract the much slower decline in apoptosis. As a result, the imbalance between both processes manifests at 30 days, agreeing with the earliest period, where there is a net loss of SMC. Since the ratio of the SMC to collagen decreases significantly, and rather drastically at 7 days after BCNR, but the content of the SMC decreases much earlier, at 3 days, which coincides with the peak in apoptosis, it may be concluded that collagen deposition is intensified after the SMC loss, and that therefore the reduction of the cellular compartment precedes the onset of fibrosis. It is the net loss of SMC that appears to trigger the first manifestation of CVOD that occurs 30 days after BCNR.

The reduction of nitrergic nerve terminals that are clearly distinguishable from the dorsal nerve and may be ascribed topologically to the cavernosal nerve, is evident as early as 1 day after BCNR. This suggests that Wallerian nerve degeneration exacerbated throughout the 45-day period is most likely responsible for the changes observed in the corpora cavernosa SMC. Most interestingly and somewhat surprisingly was the lack of changes in the content of eNOS, thereby suggesting that the endothelium is not considerably affected by BCNR. This indicates that: (i) eNOS-dependent endothelial dysfunction may not be elicited by neuropraxia and is not involved in CVOD, which appears to result mainly from corporal SMC loss and fibrosis; and (ii) in the absence of nNOS, eNOS cannot per se produce sufficient nitric oxide as to sustain the papaverine-induced production of cGMP caused by the unspecific PDE inhibition exerted by the drug [25]. However, since neither endothelial function nor eNOS activity has been determined, it is not possible to rule out a possible functional impairment of the endothelium after BCNR despite unaltered expression of eNOS.

Perhaps the most intriguing observation is the time course of iNOS induction by BCNR, which seems to follow the nNOS decrease in the nitrergic nerves but peaks at 30 days. This is long after apoptosis has reached a maximum at 3 days, thus ruling out the possibility that this cell death is triggered by nitric oxide from iNOS, a compound that is usually considered as proapoptotic [26,27]. However, there is evidence that nitric oxide can in fact be antiapoptotic according to tissue and physiological conditions [28]. Alternatively, this sustained increase of iNOS expression may be responsible for the observed reduction of the compensatory cell proliferation in the corpora after BCNR, based on the fact that both nitric oxide and cGMP are considered to be antiproliferative for the SMC in the arterial media [29]. However, this possibility appears to be ruled out by our previous results with N6-(1-iminoethyl)-L-lysine dihydrochloride (L-NIL), an inhibitor of iNOS activity [11,15]. At least at 45 days after BCNR, a steady iNOS inhibition by daily oral L-NIL significantly reduced the SMC/collagen ratio, thus suggesting that iNOS is acting by protecting the SMC or inhibiting collagen deposition, which would be in agreement with the cardioprotective effects of nitric oxide, cGMP, and iNOS on cardiomyocytes during ischemia reperfusion pre or postconditioning [30–32]. iNOS may not only be produced by smooth muscle cells, since macrophages and interstitial fibroblasts are also known to express this protein upon induction. No colocalization studies for iNOS and ASMA were performed in this work, and therefore it cannot be ruled out that iNOS synthesis in the corpora occurs also in cell types other than the smooth muscle cells. In addition, it is not surprising that a steady increase in iNOS would occur in the presence of a sustained decline in the overall content of the putative cell type where iNOS is induced, since iNOS expression is due to transcriptional stimulation, which, by a steady increase within each cell and the cumulative production of nitric oxide, can substantially exceed the rate of cell loss.

However, since CVOD and fibrosis do develop in BCNR despite the steady iNOS production, this process is apparently insufficient to counteract the factors that trigger "corporal dystrophy" (fibrosis and SMC loss), a term that we propose as analogous to skeletal muscle dystrophy.

This leads to the fundamental question regarding which factors triggered by the neuropraxia are responsible for causing corporal SMC dystrophy. The most likely is the interruption of the secretion of neurotrophins, which, in addition to their effects on neural tissue [33,34], are postulated to stimulate smooth muscle hyperplasia, particularly in the respiratory airways and the intestine [35,36]. This depletion may cause the downregulation of SMC proliferation triggered by a spontaneous defense mechanism against neuropraxia. Conversely, the induction of cytokine release, mainly TNFα and TGFβ1, that are proapoptotic and fibrotic factors and activate the proteasome ubiquitin proteolytic pathway, is a recognized feature of Wallerian degeneration [37], and it underlies at least in part the skeletal muscle atrophy subsequent to denervation [38,39]. However, the lack of neuromotor discharge and activity may also be an essential factor in this atrophy.

Irrespective of the mechanism that triggers fibrosis and SMC loss subsequent to cavernosal nerve damage, three things became obvious through this work. First, that it is the early histopathological impairment within the corpora smooth muscle that leads later to the functional impairment, CVOD. It may require a certain threshold in the smooth muscle/collagen ratio that below that threshold, the functional impairment becomes evident Therefore, in the clinical setting, an early therapeutical intervention to reduce apoptosis of the corporal SMC or sustain their initial proliferation response, would be warranted, e.g., immediately after radical prostatectomy. Second, since iNOS induction appears to be an endogenous antifibrotic and protective response on the smooth muscle, the early therapy may be based on pharmacological agents that mimic this process, such as the continuous long-term administration of PDE5 inhibitors we have studied in rats [10–13,33,40], or of nitric oxide generators [41,42], or in men for a combination of both types of compounds [43]. Such a therapeutic modality may be accompanied with neurotrophin administration, such as BDNF [34], in an attempt to restore the anabolic signals to the smooth muscle that endogenous neurotrophic factors are no longer mediating. Third, since our experimental model of BCNR, where the cavernosal nerves are both completely resected, may not be very representative of the cavernosal nerve injury that may occur with pelvic surgery, in which the nerves may only be partially damaged, it is possible that axonal regeneration, which does not seem to occur after BCNR within the time course of this study, may occur with radical pelvic surgery, particularly with the nerve sparing

procedures. As such, the treatments described above to prevent the histological changes in the corpora may also be efficacious in stimulating axonal regeneration.

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**References**

1 Levinson AW, Pavlovich CP, Ward NT, Link RE, Mettee LZ, Su LM. Association of surgeon subjective characterization of nerve sparing quality with potency following laparoscopic radical prostatectomy. J Urol 2008;179:1510–4.
2 Madeb R, Golijanin D, Knopf J, Vicente I, Erturk E, Patel HR, Joseph JV. Patient-reported validated functional outcome after extraperitoneal robotic-assisted nerve-sparing radical prostatectomy. JSLS 2007;11:443–8.
3 Ayyathurai R, Manoharan M, Nieder AM, Kava B, Soloway MS. Factors affecting erectile function after radical retropubic prostatectomy: Results from 1,620 consecutive patients. BJU Int 2008; 101:833–6.
4 Briganti A, Salonia A, Gallina A, Chun FK, Karakiewicz PI, Graefen M, Huland H, Rigatti P, Montorsi F. Management of erectile dysfunction after radical prostatectomy in 2007. World J Urol 2007; 25:143–8.
5 Luo H, Goldstein I, Udelson D. A three-dimensional theoretical model of the relationship between cavernosal expandability and percent cavernosal smooth muscle. J Sex Med 2007;4:644–5, discussion 651–5.
6 McCullough A, Woo K, Telegrafi S, Lepor H. Is sildenafil failure in men after radical retropubic prostatectomy (RRP) due to arterial disease? Penile duplex Doppler findings in 174 men after RRP. Int J Impot Res 2002;14:462–5.
7 Broderick GA. Evidence based assessment of erectile dysfunction. Int J Impot Res 1998;10(2 suppl): S64–73, discussion S77–9.
8 Nikoobakht M, Saraji A, Meysamie A. Preoperative corporal biopsy as a predictor of postoperative results in venoocclusive erectile dysfunction. Urol J 2005;2:160–4.
9 Hu WL, Hu LQ, Li SW, Zheng XM, Tian BC. Expression of transforming growth factor-beta1 in penile tissue from rats with bilateral cavernosal nerve ablation. BJU Int 2004;94:424–8.
10 Kovanecz I, Rambhatla A, Ferrini MG, Vernet D, Sanchez S, Rajfer J, Gonzalez-Cadavid NF. Chronic daily tadalafil prevents the corporal fibrosis and veno-occlusive dysfunction that occurs after cavernosal nerve resection. BJU Int 2008;101:203–10.
11 Kovanecz I, Rambhatla A, Ferrini M, Vernet D, Sanchez S, Rajfer J, Gonzalez-Cadavid NF. Long-term continuous sildenafil treatment ameliorates corporal veno-occlusive dysfunction (CVOD) induced by cavernosal nerve resection in rats. Int J Impot Res 2008;20:202–12.
12 Ferrini MG, Davila HH, Kovanecz I, Sanchez SP, Gonzalez-Cadavid NF, Rajfer J. Vardenafil prevents fibrosis and loss of corporal smooth muscle that occurs after bilateral cavernosal nerve resection in the rat. Urology 2006;68:429–35.
13 Rambhatla A, Kovanecz I, Ferrini M, Gonzalez-Cadavid NF, Rajfer J. Rationale for phosphodiesterase 5 inhibitor use post-radical prostatectomy: Experimental and clinical review. Int J Impot Res 2008;20:30–4.
14 Davila HH, Rajfer J, Gonzalez-Cadavid NF. Corporal veno-occlusive dysfunction in aging rats: Evaluation by cavernosometry and cavernosography. Urology 2004;64:1261–6.

15 Ferrini MG, Davila H, Valente EG, Gonzalez-Cadavid NF, Rajfer J. Aging-related induction of inducible nitric oxide synthase (iNOS) is vasculoprotective in the arterial media. Cardiovasc Res 2004;61:796–805.
16 Mazza ON, Angerosa M, Becher E, Toblli JE. Differences between Candesartan and Hydralazine in the protection of penile structures in spontaneously hypertensive rats. J Sex Med 2006;3:604–11.
17 Magee T, Zeller CB, Ferrini M, Davila H, Vernet D, Burnett AL, Rajfer J, González-Cadavid NFA. Protein inhibitor of NOS (PIN) is expressed in the rat and mouse penile nerves and co-localizes with penile neuronal NOS (PnNOS). Biol Reprod 2002; 68:478–88.
18 Nolazco G, Kovanecz I, Vernet D, Gelfand RA, Tsao J, Ferrini MG, Magee T, Rajfer J, Gonzalez-Cadavid NF. Effect of muscle-derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. BJU Int 2008; 101:1156–64.
19 Artaza JN, Singh R, Ferrini MG, Braga M, Tsao J, Gonzalez-Cadavid NF. Myostatin promotes a fibrotic phenotypic switch in multipotent C3H 10T1/2 cells without affecting their differentiation into myofibroblasts. J Endocrinol 2008;196: 235–49.
20 Cantini LP, Ferrini MG, Vernet D, Magee TR, Qian A, Gelfand RA, Rajfer J, Gonzalez-Cadavid NF. Profibrotic role of myostatin in peyronie's disease. J Sex Med 2008;5:1607–22.
21 Klein LT, Miller MI, Buttyan R, Raffo AJ, Burchard M, Devris G, Cao YC, Olsson C, Shabsigh R. Apoptosis in the rat penis after penile denervation. J Urol 1997;158:626–30.
22 User HM, Hairston JH, Zelner DJ, McKenna KE, McVary KT. Penile weight and cell subtype specific changes in a post-radical prostatectomy model of erectile dysfunction. J Urol 2003;169:1175–9.
23 Lysiak JJ, Yang SK, Klausner AP, Son H, Tuttle JB, Steers WD. Tadalafil increases Akt and extracellular signal-regulated kinase 1/2 activation, and prevents apoptotic cell death in the penis following denervation. J Urol 2008;179:779–85.
24 Podlasek CA, Meroz CL, Tang Y, McKenna KE, McVary KT. Regulation of cavernous nerve injury-induced apoptosis by sonic hedgehog. Biol Reprod 2007;76:19–28.
25 Maggi M, Filippi S, Ledda F, Magini A, Forti G. Erectile dysfunction: From biochemical pharmacology to advances in medical therapy. Eur J Endocrinol 2000;143:143–54.
26 Erusalimsky JD, Moncada S. Nitric oxide and mitochondrial signaling: From physiology to pathophysiology. Arterioscler Thromb Vasc Biol 2007; 27:2524–31.
27 Szabó C, Ischiropoulos H, Radi R. Peroxynitrite: Biochemistry, pathophysiology and development of therapeutics. Nat Rev Drug Discov 2007;6:662–80.
28 Choi BM, Pae HO, Jang SI, Kim YM, Chung HT. Nitric oxide as a pro-apoptotic as well as anti-apoptotic modulator. J Biochem Mol Biol 2002;35:116–26.
29 Muhs A, Heublein B, Schletter J, Herrmann A, Rüdiger M, Sturm M, Grust A, Malms J, Schrader J, von der Leyen HE. Preclinical evaluation of inducible nitric oxide synthase lipoplex gene therapy for inhibition of stent-induced vascular neointimal lesion formation. Hum Gene Ther 2003;14:375–83.
30 Cohen MV, Downey JM. Cardioprotection: Spotlight on PKG. Br J Pharmacol 2007;152:833–4.
31 Burley DS, Ferdinandy P, Baxter GF. Cyclic GMP and protein kinase-G in myocardial ischaemia-reperfusion: Opportunities and obstacles for survival signaling. Br J Pharmacol 2007;152:855–69.
32 Li Q, Guo Y, Tan W, Ou Q, Wu WJ, Sturza D, Dawn B, Hunt G, Cui C, Bolli R. Cardioprotection afforded by inducible nitric oxide synthase gene therapy is mediated by cyclooxygenase-2 via a nuclear factor-kappa B dependent pathway. Circulation 2007;116:1577–84.
33 Lagoda G, Jin L, Lehrfeld TJ, Liu T, Burnett AL. FK506 and sildenafil promote erectile function recovery after cavernous nerve injury through antioxidative mechanisms. J Sex Med 2007;4:908–16.
34 Lin G, Bella AJ, Lue TF, Lin CS. Brain-derived neurotrophic factor (BDNF) acts primarily via the JAK/STAT pathway to promote neurite growth in the major pelvic ganglion of the rat: Part 2. J Sex Med 2006;3:821–7; discussion 828–9.
35 Rochlitzer S, Nassenstein C, Braun A. The contribution of neurotrophins to the pathogenesis of allergic asthma. Biochem Soc Trans 2006;34: 594–9.
36 Groneberg DA, Rabe KF, Fischer A. Novel concepts of neuropeptide-based drug therapy: Vasoactive intestinal polypeptide and its receptors. Eur J Pharmacol 2006;533:182–94.
37 Palin K, Cunningham C, Forse P, Perry VH, Platt N. Systemic inflammation switches the inflammatory cytokine profile in CNS Wallerian degeneration. Neurobiol Dis 2008;30:19–29.
38 Midrio M. The denervated muscle: Facts and hypotheses. A historical review. Eur J Appl Physiol 2006;98:1–21.
39 Jagoe RT, Goldberg AL. What do we really know about the ubiquitin-proteasome pathway in muscle atrophy? Curr Opin Clin Nutr Metab Care 2001; 4:183–90.
40 Ferrini MG, Kovanecz I, Sanchez S, Vernet D, Davila HH, Rajfer J, Gonzalez-Cadavid NF. Long-term continuous treatment with sildenafil ameliorates aging-related erectile dysfunction and the underlying corporal fibrosis in the rat. Biol Reprod 2007;76:915–23.

41 Vernet D, Ferrini MG, Valente E, Magee TR, Bou-Gharios G, Rajfer J, Gonzalez-Cadavid NF. Effect of nitric oxide on the differentiation of fibroblasts into myofibroblasts in the Peyronie's fibrotic plaque and in its rat model. Nitric Oxide 2002;7: 262–76.
42 Valente EG, Ferrini MG, Vernet D, Qian A, Rajfer J, Gonzalez-Cadavid NF. PDE L-arginine and PDE inhibitors counteract fibrosis in the Peyronie's fibrotic plaque and related fibroblast cultures. Nitric Oxide 2003;9:229–44.
43 Rajfer J, Gore JL, Kaufman J, Gonzalez-Cadavid N. Case report: Avoidance of palpable corporal fibrosis due to priapism with upregulators of nitric oxide. J Sex Med 2006;3:173–6.

# Mechanisms of Penile Fibrosis

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**ABSTRACT**

***Introduction.*** Penile fibrosis has been conceptually identified with the plaque that develops in the tunica albuginea in Peyronie's disease (PD), or with localized processes induced in the corpora cavernosa by ischemic or traumatic events. Recently, it has been proposed that a diffuse, progressive, and milder intracorporal fibrosis, which affects also the media of the penile arteries, is responsible for vasculogenic erectile dysfunction (ED) associated with aging, smoking, diabetes, hypertension, and post-radical prostatectomy. These processes differ in etiology, time course, target cells, and treatment, but have many features in common.
***Aim.*** To review the literature pertaining to fibrosis in the penis, related to PD and ED.
***Methods.*** PubMed search for pertinent publications mainly during 2001–2008.
***Results.*** This review focuses initially on PD and then deals with studies on ED in animal and cell culture models, discussing some of the pathophysiological similarities between tunical fibrosis in PD and corporal fibrosis in corporal veno-occlusive dysfunction (CVOD), and emerging therapeutic strategies. The role of profibrotic factors, the excessive deposit of collagen fibers and other extracellular matrix, the appearance of a synthetic cell phenotype in smooth muscle cells or the onset of a fibroblast–myofibroblast transition, and in the case of the corporal or penile arterial tissue the reduction of the smooth muscle cellular compartment, are discussed. This histopathology leads either to localized plaques or nodules in penile tissues, or to the diffuse fibrosis causing impairment of tissue compliance that underlies CVOD and arteriogenic ED. The antifibrotic role of the sustained stimulation of the nitric oxide/cyclic guanosine monophosphate pathway in the penis and its possible relevance to exogenous and endogenous stem cell differentiation is also briefly presented.
***Conclusions.*** Fibrotic processes in penile tissues share a similar cellular and molecular pathophysiology and common endogenous mechanisms of defense that have inspired novel pharmacological experimental approaches. **Gonzalez-Cadavid NF. Mechanisms of penile fibrosis. J Sex Med 2009;6(suppl 3):353–362.**

***Key Words.*** Erectile Dysfunction; Corporal Veno-Occlusive Dysfunction; Inducible Nitric Oxide Synthase

## Introduction

The topic of urogenital fibrosis is dominated by the considerable significance of tubulointerstitial fibrosis and glomerulosclerosis in chronic kidney disease, mainly diabetic nephropathy [1,2], and of postsurgical adherences [3], but very little attention has been focused on fibrotic processes in other urogenital disorders. Until recently, penile fibrosis was assumed to be limited to the Peyronie's disease (PD) plaque in the tunica albuginea [4] or to the comparatively rare events subsequent to tissue insults such as intracorporeal injection or prolonged priapism [5]. However, in the last few years, it has become evident that fibrosis of the corpora cavernosa and the media of penile arteries, involving loss of smooth muscle cells (SMC), is a highly prevalent process that underlies most cases of vasculogenic erectile dysfunction (ED) (see e.g., [6–10]). Therefore, the study of fibrosis may provide a unifying view on the two most prevalent disorders affecting the penis, even if located in different tissues. This presentation discusses some selected results, focusing on the contributions from our group at the University of California, Los Angeles.

- **Excessive accumulation and disorganization of collagen fibers and other extracellular matrix, with reduction of cell number and, usually, myofibroblast accumulation**
- **Diffuse or localized in most organs; also in abnormal wound healing**
- **Leads to tissue and organ dysfunction**
- **Subsequent to acute injury or chronic inflammation, with release of cytokines (transforming growth factor beta 1), plasminogen activator inhibitor 1, and oxidative stress (reactive oxygen species)**

**Figure 1** Main features of the fibrotic process.

The excessive deposition of collagen and extracellular matrix (ECM) accompanied by the loss of functional cells that characterize tissue fibrosis, is due in some cases to the appearance and accumulation of myofibroblasts or in other cases to the switch to a synthetic phenotype producing ECM of the original cell components, such as fibroblasts and/or SMC in the penis (Figure 1). The main factor in eliciting these cellular alterations is an insult to the tissue, be it: (i) acute and localized, in a specific site in the tunica albuginea in PD [11,12]; (ii) acute and diffuse throughout the corpora such as in cavernosal nerve damage after radical prostatectomy [8,13–19]; or (iii) chronic and also diffuse throughout the corpora and the penile arteries wall such as in aging, diabetes, and heavy smoking [9,10,20–26]. The corporal and arterial alterations lead to corporal veno-occlusive dysfunction (CVOD), the most prevalent form of vasculogenic ED [27] that can be measured in the rat by cavernosometry [28]. The initial insult to any of these penile tissues results in the release of profibrotic factors, mainly transforming growth factor beta 1 (TGFβ1), plasminogen activator inhibitor 1 (PAI-1), and reactive oxygen species (ROS) leading to oxidative stress, that may be in some cases exacerbated by chronic inflammation. This is remarkably similar to what occurs in the more widely studied diffuse fibrosis in kidney, lung, liver, and skin, or the localized processes in abnormal wound healing leading to scars or myocardial infarction [1,29–33].

### Fibrosis of the Tunica Albuginea in Peyronie's Disease

The main culprit of fibrosis in PD is the myofibroblast, that is key for normal wound healing but is eliminated by apoptosis after the tissue is healed; when this does not occur, fibrosis develops [33]. It is likely that the same occurs in vascular and corporal fibrosis, but the difficulties in differentiating myofibroblasts from SMC have not yet allowed their proper identification. Experimental studies in PD were based in the combination of two human models (the PD plaque and its normal tunical counterpart, and cell cultures derived from these tissues) and two animal models where the lesion was elicited by either TGFβ1 or fibrin [34] (Figure 2). They, together with those of Mulhall's group [35,36], have shown the appearance and persistence of myofibroblasts in PD and have clarified some of their features.

In those earlier studies, we showed that oxidative stress and PAI-1 increase in the PD plaque, and very important, that the inducible nitric oxide synthase (iNOS) isoform that produces nitric oxide (NO) and is usually considered a defense mechanism against infection or cancer and is associated with inflammation, is remarkably increased in the human and animal plaques [11,37–43]. This was illustrated by the simultaneous increase of iNOS and an antioxidant enzyme in the fibroblasts of the human PD plaque evaluated by immunohistochemistry/quantitative image analysis (QIA) and by the reduction of the PD-like plaque stained for collagen by Masson trichrome and QIA. Moreover, the plaques are not an irreversible dead-end but are in a constant state of molecular and cellular turnover, as determined by DNA microarrays, involving a balance between fibrotic and antifibrotic mechanisms [44,45] (Figure 3).

The accumulation of myofibroblasts in the fibrotic PD plaque was detected, as compared with the normal tunica albuginea, by immunohistochemical staining and quantitative image analysis for α-smooth muscle actin (ASMA), a marker of both myofibroblasts and SMC, and also by Western blot. Cell cultures followed by both types of assays confirmed these findings [35,36,39]. The spontaneous chronic induction of iNOS, a leitmo-

- **PD plaque and tunica albuginea tissues in men**
- **Fibroblast cultures from tissues**
- **First rat model (1997, UCSF): PD-like plaque in the rat tunica by transforming growth factor beta 1 (TGFβ1) peptide**
- **Second rat model (2003, UCLA): fibrin injection in the rat tunica, eliciting TGFβ1**

**Figure 2** Models for studying fibrosis in Peyronie's disease (PD).

- **Excessive deposition of collagen fibers, oxidative stress, and persistence of myofibroblasts, seen in other fibrosis (2002-3, UCLA)**
- **Spontaneous expression of inducible nitric oxide synthase leading to a sustained output of nitric oxide protecting against oxidative stress and fibrosis (UCLA, 2002-3)**
- **The PD plaque is under a steady cellular and molecular turnover (2003-5, UCLA)**

**Figure 3** The PD plaque as a model of localized penile fibrosis.

tif of other fibrosis, was postulated by us to be an antifibrotic mechanism, based essentially on the exacerbation of fibrosis, myofibroblast production, and oxidative stress in the rat PD-like plaques by blockade of iNOS activity with L-N-(1-iminoethyl)-lysine acetate (L-NIL), and conversely of their reduction by gene transfer of iNOS complementary DNA (cDNA) to the plaque [38,39,42] (Figure 4).

This led us to postulate that the steady production of NO from iNOS quenches ROS, producing peroxynitrite that reduces the levels of profibrotic ROS, and on the other hand NO from iNOS causes myofibroblast apoptosis. NO, as well as its product, cyclic guanosine monophosphate (cGMP), also inhibits collagen synthesis directly as demonstrated in fibroblast cultures from the normal human tunica [39]. The pharmacological implication was that phosphodiesterase type 5 (PDE5) inhibitors, by elevating the content of cGMP in penile tissues, would reverse the plaque and combat myofibroblast accumulation. First with sildenafil, and later with vardenafil given from the induction of the plaque and even after it was formed, we showed by immunocytochemistry/QIA that ASMA staining was considerably reduced, as well as plaque size and oxidative stress [46–48].

TGFβ1 may be the main, but is not the only profibrotic factor involved in the development

- **iNOS spontaneous induction in the rat PD-like plaque as in the human PD (2000, Tulane; 2002, UCLA)**
- **iNOS inhibition in the rat models increases fibrosis, myofibroblasts, and oxidative stress (2002-3, UCLA)**
- **iNOS induction by gene transfer inhibits these processes (2004-5, UCLA)**

**Figure 4** Inducible nitric oxide synthase (iNOS) is an antifibrotic factor in Peyronie's disease (PD).

**Basic premise: shift tissue turnover to:**

- **predominance of collagen breakdown over synthesis**
- **predominance of myofibroblast apoptosis over proliferation**
- **switch off myofibroblast and osteoblast differentiation from stem cells**

**Figure 5** Novel pharmacological strategies for PD.

of the plaque. Very recently we showed by immunohistochemistry/QIA that another member of this superfamily that comprises activins, myostatin or GDF-8, is considerably expressed in the human PD plaque, mainly in myofibroblasts [49]. By using cDNA for myostatin, we demonstrated in the rat model that myostatin per se induced a plaque or exacerbated the one produced by TGFβ1 as shown by Masson trichrome/QIA. However, this effect was ancillary to the action of TGFβ1 and not essential, as blocking myostatin production with a small hairpin RNA (shRNA) against this protein did not affect the size of the TGFβ1-induced plaque.

These studies, and particularly the concept that fibrosis is not irreversible, that the key target cell is the myofibroblast, and that it appears to originate from stem cells that are present in the tunica albuginea and originate other cell lines, justify to investigate novel strategies for the pharmacological therapy of PD to block these processes, particularly the modulation of stem cell or progenitor cells in the tunica [25,43] (Figure 5).

### Fibrosis of the Corporal Tissue and Arterial Media Associated with Vasculogenic ED

As stated initially, fibrosis of the corporal smooth muscle and the penile arteries media has emerged as the predominant underlying cause of ED caused by the most diverse risk factors [5–10,13–26] (Figure 6). iNOS plays here the same antifibrotic role occurring in PD, and even in the diabetic vagina [50] that can be exploited pharmacologically with similar approaches to the ones used for PD [51]. This is shown by the fact that corporal fibrosis measured by Masson trichrome/QIA for the SMC/collagen ratio (the inverse ratio of the one used for PD where collagen/extracellular ratio was measured) and by hydroxyproline determinations in penile tissue hydrolyzates, presents during aging of the iNOS knockout (ko) mouse where iNOS expression is genetically abrogated, already in the young adult stage (8 months of age) [52].

- **Most common form of ED is corporal veno-occlusive dysfunction (CVOD)**
- **Associated with aging, diabetes, cavernosal nerve damage, and possibly low testosterone**
- **CVOD is caused by fibrosis of the smooth muscle of the corpora cavernosa and the media of the penile arteries**
- **Inducible nitric oxide synthase, as in Peyronie's disease, plays an antifibrotic role that can be mimicked pharmacologically**

**Figure 6** The predominant underlying cause of erectile dysfunction (ED) is diffuse corporal tissue fibrosis.

This continues throughout the life span, whereas in the wild type animal there is a progressive but mild fibrosis peaking at 20 months of age. A similar exacerbation of fibrosis by iNOS deletion is seen in the iNOS ko mouse rendered diabetic by streptozotocin injection [53].

The corporal fibrosis, denoted in the trabecular tissue of the corpora containing the SMC, by the decrease of the SMC/collagen ratio and of the ASMA positive staining, is also evident in the experimental rat, specifically in the models for aging, diabetes, and castration as compared with their respective controls [9,20–23,25,28]. These histological alterations underlie CVOD, as measured by dynamic infusion cavernosometry particularly in terms of the drop rate.

As in the case of PD, long-term continuous treatment for 2 months of aged rats (20-month old) with an oral PDE5 inhibitor, in this case sildenafil, in the drinking water corrects CVOD as shown by cavernosometry after a 3-day washout [9], and the same occurs with a peroxisome proliferator activated receptor γ (PPARγ) agonist, pioglitazone, that presumably acts via an antioxidant and anti-inflammatory mechanism on both aged and diabetic rats [22,23]. This is accompanied, as expected, by a substantial increase of corporal SMC determined by ASMA immunohistochemistry/QIA.

The CVOD occurs also in adult rats after bilateral cavernosal nerve resection (BCNR) that mimics the cavernosal nerve damage in men after non-nerve sparing radical prostatectomy, and this develops gradually as a consequence of the neuropraxia, with is first manifestation seen between 15 and 30 days after BCNR. This functional alteration is preceded by a significant decrease in the SMC/collagen ratio or the SMC content in the corpora at 7 and 3 days, respectively, after BCNR [8,16–19]. The loss of SMC appears to result from a predominance of apoptosis, determined by the TUNEL procedure, over the initial bout of cell proliferation, measured by immunohistochemistry for proliferating cell nuclear antigen, that aims to counteract the apoptosis already seen as early as 1 day after BCNR [19]. There is a sustained increase of iNOS that peaks at 30 days.

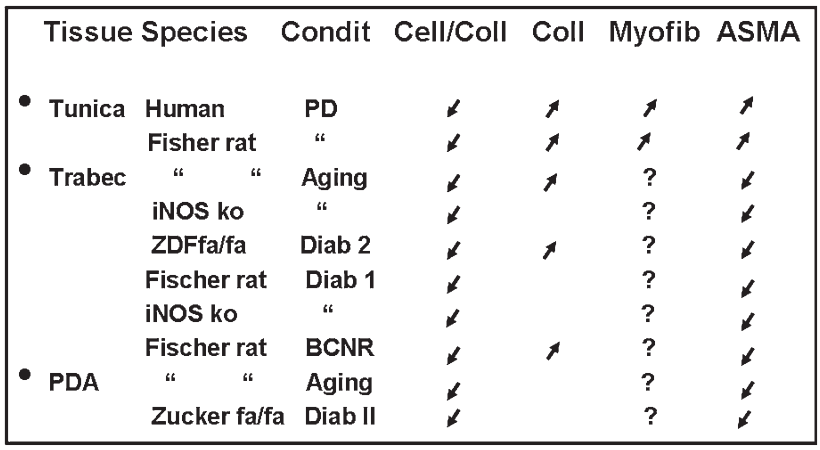
Again, as in the case of the aged rat and PD, the three PDE5 inhibitors, tadalafil, sildenafil, and vardenafil given as a sustained oral administration for 45 days immediately after BCNR prevent CVOD, while restoring the normal SMC/collagen ratio estimated by Masson/QIA to about the same extent [8,16–18]. These results have been replicated using a milder nerve injury procedure in the cavernosal nerve crush injury model, where a daily treatment with sildenafil for 28 days resulted in the protection of the SMC/collagen ratio and the endothelium, and the improvement of the electrical field stimulation (EFS) response in a time- and dose-dependent fashion [54]. Interestingly, the three PDE5 inhibitors administered orally for short periods (up to 36 hours) to normal intact rats also increased heme oxygenase activity, which would suggest that they may reduce oxidative stress and hence pro-fibrotic ROS, but this has not yet been tested in any of the corporal fibrosis models [55].

The preservation of the SMC content in the corpora may also be exerted by antiapoptotic effects of other agents such as growth differentiation factor 5 (GDF-5) or the immunophilin ligand FK506 in the cavernosal nerve injury model [56,57]. Similarly, insulin-like growth factor 1 (IGF-1) given to aged rats for 4 or 8 weeks increased the percentage of corporal SMC and stimulated the erectile response to EFS [58].

The SMC fibrosis is not restricted to the corpora, because during aging it is also seen in the media of the penile arteries, in this case the penile dorsal artery (PDA) [10]. iNOS is also overexpressed in the aged arteries and its blockade leads to an increase in fibrosis measured by SMC/collagen ratio. An identical loss of SMC and increase in apoptosis occurs in the PDA and the aorta in the ZDF rat, a model for type 2 diabetes [24].

### Integrated View of Fibrosis Mechanisms in Penile Tissues

Figure 7 compiles these results and shows that in the tunica adventitia, the corpora cavernosa, and the penile arterial media, the decrease of the cellular/collagen ratio, and the increase of total

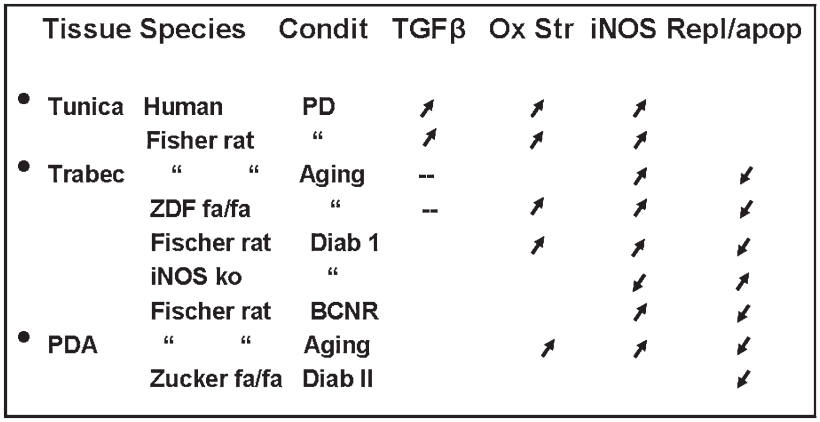



ASMA = α-smooth muscle actin; BCNR = bilateral cavernosal nerve resection; C = collagen; Condit = condition; Diab = diabetes; Myofib = myofibroblast; iNOS = inducible nitric oxide synthase; PD = Peyronie's disease; PDA = penile dorsal artery; Trabec = corporal trabecular tissue.

**Figure 7** Cellular/extracellular matrix balance in penile fibrosis.

collagen when this was measured, are the common denominators, irrespective of the type of tissue, the animal model, or the pathological outcome. ASMA, a dual myofibroblast/SMC marker, is increased when there is myofibroblast accumulation in the tunica in PD, but reduced when the SMC are lost in the corpora or the arteries in the models of CVOD. However, because of the duality of ASMA as a marker, no information is as yet available on the presence of myofibroblasts in the corporal or arterial smooth muscle, as they cannot be discriminated with ASMA from SMC. Figure 8, in turn, shows iNOS induction to be the other common denominator, whereas apoptosis is seen in all cases of corporal fibrosis. On the other hand, TGFβ1 induction is restricted to PD or the vagina [11,38,50] as it was not seen as a significant process in the penile corpora cavernosa during aging, diabetes, or BCNR. Oxidative stress occurs mainly in PD [11,38] but also in chronic corporal fibrosis in diabetes [23].

The overall lesson from all these models is the severe alteration of the cellular/ECM balance observed in the tunical fibroblasts and the corporal and arterial SMC, with myofibroblasts being clearly identified in the PD plaque [37,41,47], but




apop = apoptosis; BCNR = bilateral cavernosal nerve resection; Condit = condition; Diab = diabetes; Ox Str = oxidative stress; iNOS = inducible nitric oxide synthase; ko = knockout mouse; PD = Peyronie's disease; PDA = penile dorsal artery; Repl = replication; TGFβ = transforming growth factor beta; Trabec = corporal trabecular tissue.

**Figure 8** Fibrotic/antifibrotic balance in penile tissues.

| | Tissue | Species | Cond | NO + | NO - | PDE5i |
|---|---|---|---|---|---|---|
| • | Tunica | Fisher rat | PD | L-arg<br>iNOS cDNA | L-NIL | varden<br>silden |
| • | Trabec | " " | Aging | | | silden |
| | | ZDF fa/fa | Diab 2 | Molsido | L-NIL | silden |
| | | iNOS ko | " | Molsido | L-NIL | |
| | | Fischer rat | BCNR | | L-NIL | silden<br>tadal<br>varden |
| • | PDA | " " | Aging | | L-NIL | |

BCNR = bilateral cavernosal nerve resection; cDNA = complementary DNA; Condit = condition; Diab = diabetes; iNOS = inducible nitric oxide synthase; ko = knockout mouse; NO+ = NO generator; NO- = iNOS inhibitor; PD = Peyronie's disease; PDA = penile dorsal artery; PDE5i = phosphodiesterase type 5 inhibitors; silden = sildenafil; tadal = tadalafil; Trabec = corporal trabecular tissue; varden = vardenafil.

**Figure 9** Nitric oxide (NO)/cyclic guanosine monophosphate modulation of the fibrotic phenotype in penile tissues.

not as yet in the SMC tissues, as this may be obscured by the overall reduction in SMC. Similarly, the fibrotic process, indicated by TGFβ1 in some cases, oxidative stress and the proliferation/apoptotic ratio, is counteracted by the antifibrotic induction of iNOS and its products, NO and cGMP (see e.g., [34,41,51]). This is supported by the fact that blocking the iNOS endogenous mechanism of defense with L-NIL exacerbates fibrosis [9,10,38,39,42,52,53] (Figure 9). Also, inhibiting the action of members of the TGFβ superfamily, such as with shRNA for myostatin [49] or the TGFβ1 blocker decorin ameliorates penile fibrosis.

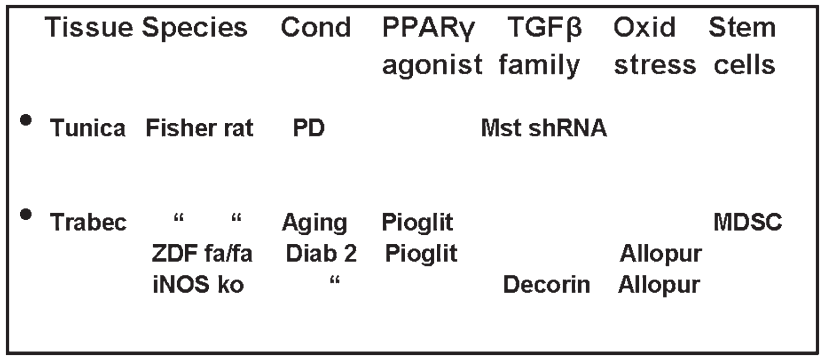
### Other Novel Therapeutic Approaches to Prevent Fibrotic Processes in Penile Tissues

An emerging approach to treat corporal fibrosis is the replacement of the lost SMC by implanted stem cells (that can also be engineered ex vivo to express antifibrotic genes [59,60] (Figure 10). We recently showed that stem cells isolated from the skeletal muscle of mice can be implanted into the rat corpora cavernosa of old rats with ED and generate SMC [25]. By undergoing this conversion, the muscle-derived stem cells (MDSC) corrected the ED in the aged rats after even 4 weeks, as measured by electrical field stimulation of the cavernosal nerve. We are now studying the ex vivo gene engineering of MDSC with a series of genes directed by regulable promoters aimed to act via different antifibrotic mechanisms: elevation of antifibrotic nitric oxide or cGMP levels (by iNOS cDNA or PDE5 shRNA) [61], or counteraction of the pro-fibrotic myostatin (by myostatin shRNA) [49,62,63]. The blockade of the Smad pathway, which is a common downstream signaling mechanism for both TGFβ1 or myostatin, is also a potential antifibrotic strategy, as upregulation of the expression of TGFβ1 and phospho-activation of the Smad pathway was shown to occur in the penis of the rat with streptozotocin-induced diabetes (a model for type 1 diabetes) [64]. Another promising approach is via the modulation of metalloproteinase expression by overexpression with the respective cDNA [65].

However, perhaps the most promising and novel approach is the pharmacological modulation of endogenous stem cells in the penis to produce SMC and to block myofibroblast generation. These cells have been identified in the rat penile tunica albuginea and trabecular tissue by immunohistochemistry and immunofluorescence for stem cell markers such as Sca1, and in

- **Oxidative stress → cytokine liberation → chronic inflammation → fibrosis → loss of functional cells and tissue compliance**
- **Cell replacement by transplanted stem cells and/or pharmacological modulation of endogenous stem cells**
- **Ex vivo engineering for modulating stem cell lineage commitment**

**Figure 10** Stem cells in the therapy of tissue fibrosis and remodeling.




Allopur = allopurinol; Cond = condition; Diab = diabetes; iNOS = inducible nitric oxide synthase; Mst = myostatin; Oxid = oxidative; PD = Peyronie's disease; Pioglit = pioglitazone; PPARy = peroxisome proliferator activated receptor gamma; shRNA = small hairpin RNA; TGFB = transforming growth factor beta; Trabec = corporal trabecular tissue.

**Figure 11** Other modulation of the fibrotic phenotype in penile tissues.

cell cultures from the human tunica [25,43]. The latter cultures have the ability in vitro to generate different cell lineages. We have identified by DNA microarrays another stem cell marker in the MDSC used for the previous experiments of stem cell implantation in the penis and also for vaginal regeneration [66]. This is Oct-4, an embryonic stem cell marker, used recently to program, together with three other embryonic stem cell genes, fibroblasts from adult skin into a multipotent stage similar to embryonic stem cells [67]. This has allowed us to identify putative stem cells in penile tissues that express Oct-4 and may be or may be not identical to the Sca1+, CD34+ cells previously detected by us [25,43], or the embryonic-like stem cells detected in a variety of adult tissues [68]. These endogenous stem cells may be good candidates for antifibrotic pharmacological modulation, particularly with agents belonging to the NO/cGMP and TGFβ1 pathway. We believe that this approach is not far-fetched and actually more feasible than regular gene and stem cell therapy for combating penile fibrosis and restoring the normal cell compartments in both the corpora and the tunica.

- **Inhibit myofibroblast generation, replenish smooth muscle cells, counteract fibrotic phenotype, by:**
- **Nitric oxide generators**
- **Phosphodiesterase type 5 inhibitors**
- **Anti-transforming growth factor beta family/smad agents**
- **Cell differentiation master genes**

**Figure 12** Pharmacological modulation of endogenous stem cell differentiation in the penis to counteract fibrosis.

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*Conflict of Interest.* None declared.

## References

1 Qian Y, Feldman E, Pennathur S, Kretzler M, Brosius FC 3rd. From fibrosis to sclerosis: Mechanisms of glomerulosclerosis in diabetic nephropathy. Diabetes 2008;57:1439–45.
2 Toblli JE, Ferrini MG, Cao G, Vernet D, Angerosa M, Gonzalez-Cadavid NF. Antifibrotic effects of pioglitazone on the kidney in a rat model of type 2 diabetes mellitus. Diabetes submitted abstract: National Kidney Foundation (NKF) 2008 Spring Clinical Meetings, Dallas, Texas.
3 Lee JY, Stenzel W, Impekoven P, Theisohn M, Stützer H, Löhr M, Reithmeier T, Ernestus RI, Ebel H, Klug N. The effect of mitomycin C in reducing epidural fibrosis after lumbar laminectomy in rats. J Neurosurg Spine 2006;5:53–60.
4 Gonzalez-Cadavid NF, Rajfer J. Mechanisms of disease: New insights into the cellular and molecular pathology of Peyronie's disease. Nat Clin Pract Urol 2005;2:291–8.
5 Burnett AL, Bivalacqua TJ. Priapism: Current principles and practice. Urol Clin North Am 2007; 34:631–42.

6 Iacono F, Giannella R, Somma P, Manno G, Fusco F, Mirone V. Histological alterations in cavernous tissue after radical prostatectomy. J Urol 2005;173: 1673–6.
7 Schwartz EJ, Wong P, Graydon RJ. Sildenafil preserves intracorporeal smooth muscle after radical retropubic prostatectomy. J Urol 2004;171:771–4.
8 Rambhatla A, Kovanecz I, Ferrini M, Gonzalez-Cadavid NF, Rajfer J. Rationale for phosphodiesterase 5 inhibitor use post-radical prostatectomy: Experimental and clinical review. Int J Impot Res 2008;20:30–4.
9 Ferrini MG, Kovanecz I, Sanchez S, Vernet D, Davila HH, Rajfer J, Gonzalez-Cadavid NF. Long-term continuous treatment with sildenafil ameliorates aging-related erectile dysfunction and the underlying corporal fibrosis. Biol Reprod 2007;76: 915–23.
10 Ferrini MG, Davila H, Valente EG, Gonzalez-Cadavid NF, Rajfer J. Aging-related induction of inducible nitric oxide synthase (iNOS) is vasculoprotective in the arterial media. Cardiovasc Res 2004;61:796–805.
11 Davila H, Ferrini M, Rajfer J, Gonzalez-Cadavid NF. Fibrin induction of a Peyronie's-like plaque in the rat penile tunica albuginea. A new model for Peyronie's disease. Br J Urol 2003;91:830–8.
12 Somers KD, Dawson DM. Fibrin deposition in Peyronie's disease plaque. J Urol 1997;157:311–5.
13 Klein LT, Miller MI, Buttyan R, Raffo AJ, Burchard M, Devris G, Cao YC, Olsson C, Shabsigh R. Apoptosis in the rat penis after penile denervation. J Urol 1997;158:626–30.
14 User HM, Hairston JH, Zelner DJ, McKenna KE, McVary KT. Penile weight and cell subtype specific changes in a post-radical prostatectomy model of erectile dysfunction. J Urol 2003;169:1175–9.
15 Leungwattanakij S, Bivalacqua TJ, Usta MF, Yang DY, Hyun JS, Champion HC, Abdel-Mageed AB, Hellstrom WJ. Cavernous neurotomy causes hypoxia and fibrosis in rat corpus cavernosum. J. Androl 2003;24:239–49.
16 Ferrini MG, Davila H, Kovanecz I, Sanchez S, Gonzalez-Cadavid NF, Rajfer J. Long-term continuous treatment with vardenafil prevents fibrosis and preserves smooth muscle content in the rat corpora cavernosa after bilateral cavernosal nerve transaction. Urology 2006;68:429–35.
17 Kovanecz I, Rambhatla A, Ferrini MG, Vernet D, Sanchez S, Rajfer J, Gonzalez-Cadavid NF. Long term sildenafil treatment ameliorates corporal veno-occlusive dysfunction (CVOD) induced by cavernosal nerve resection in rats. Int J Impot Res 2007;100:867–74.
18 Kovanecz I, Rambhatla A, Ferrini MG, Vernet D, Sanchez S, Rajfer J, Gonzalez-Cadavid NF. Chronic daily tadalafil prevents the corporal fibrosis and veno-occlusive dysfunction (CVOD) that occurs following cavernosal nerve resection in the rat. BJU Int 2008;101:203–10.
19 Ferrini MG, Kovanecz I, Sanchez S, Vernet D, Umeh C, Rajfer J, Gonzalez-Cadavid NF. Fibrosis and loss of smooth muscle in the corpora cavernosa precede corporal veno-occlusive dysfunction (CVOD) induced by experimental cavernosal nerve damage in the rat. J Sex Med 2008 (in press).
20 Garban H, Vernet D, Freedman A, Rajfer J, Gonzalez-Cadavid NF. Effect of aging on nitric oxide-mediated penile erection in the rat. Am J Physiol 1995;268:H467–75.
21 Ferrini M, Magee TR, Vernet D, Rajfer J, Gonzalez-Cadavid NF. Aging-related expression of inducible nitric oxide synthase (iNOS) and markers of tissue damage in the rat penis. Biol Reprod 2001; 64:974–82.
22 Kovanecz I, Ferrini MG, Davila HH, Rajfer J, Gonzalez-Cadavid NF. Pioglitazone ameliorates penile corpora veno-occlusive dysfunction (CVOD) in the aged rat. BJU Int 2007;100:867–74.
23 Kovanecz I, Ferrini MG, Vernet D, Nolazco G, Rajfer J, Gonzalez-Cadavid NF. Pioglitazone prevents corporal veno-occlusive dysfunction (CVOD) in a rat model of type 2 diabetes mellitus. BJU Int 2006;98:116–24.
24 Kovanecz I, Nolazco G, Ferrini MG, Toblli JE, Heydarkhan S, Vernet D, Rajfer J, Gonzalez-Cadavid NF. Early onset of fibrosis within the arterial media in a rat model of type 2 diabetes mellitus exhibiting erectile dysfunction. BJU Int 2008 (in press).
25 Nolazco G, Kovanecz I, Vernet D, Ferrini M, Gelfand B, Tsao J, Magee T, Rajfer J, Gonzalez-Cadavid NF. Effect of muscle derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. BJU Int 2008; 101:1156–64.
26 Dávila HH, Ferrini MG, Magee TR, Rajfer J, González-Cadavid NF. Detrimental effect of cigarette smoking on erectile dysfunction in the rat is contingent to diabetes as co-morbidity. J Urol 2004;171:374 (#1420).
27 Broderick GA. Evidence based assessment of erectile dysfunction. Int J Impot Res 1998;10: (2 suppl):S64–73; discussion S77–9.
28 Davila HH, Rajfer J, Gonzalez-Cadavid NF. Corporal veno-occlusive dysfunction in the aging rat. Evaluation by cavernosometry and cavernosography. Urology 2004;64:1261–6.
29 Aneja A, Tang WH, Bansilal S, Garcia MJ, Farkouh ME. Diabetic cardiomyopathy: Insights into pathogenesis, diagnostic challenges, and therapeutic options. Am J Med 2008;121:748–57.
30 Sun Y. Oxidative stress and cardiac repair/remodeling following infarction. Am J Med Sci 2007;334:197–205.
31 Svegliati-Baroni G, De Minicis S, Marzioni M. Hepatic fibrogenesis in response to chronic liver

injury: Novel insights on the role of cell-to-cell interaction and transition. Liver Int 2008;28:1052–64.
32 Nagai S, Handa T, Kim D. Pharmacotherapy in patients with idiopathic pulmonary fibrosis. Expert Opin Pharmacother 2008;9:1909–25.
33 Darby IA, Hewitson TD. Fibroblast differentiation in wound healing and fibrosis. Int Rev Cytol 2007;257:143–79.
34 Gonzalez-Cadavid NF, Rajfer J. Experimental models for Peyronie's disease. Implications for therapy. J Sex Med 2008 (in press).
35 Mulhall JP, Nicholson B, Pierpaoli S, Lubrano T, Shankey TV. Chromosomal instability is demonstrated by fibroblasts derived from the tunica of men with Peyronie's disease. Int J Impot Res 2004;16: 288–93.
36 Mulhall JP, Branch J, Lubrano T, Shankey TV. Perturbation of cell cycle regulators in Peyronie's disease. Int J Impot Res 2001;13(5 suppl):S21–8.
37 Bivalacqua TJ, Diner EK, Novak TE, Vohra Y, Sikka SC, Champion HC, Kadowitz PJ, Hellstrom WJ. A rat model of Peyronie's disease associated with a decrease in erectile activity and an increase in inducible nitric oxide synthase protein expression. J Urol 2000;163:1992–8.
38 Ferrini MG, Vernet D, Magee TR, Shahed A, Quian A, Rajfer J, Gonzalez-Cadavid NF. Antifibrotic role of inducible nitric oxide synthase (iNOS). Nitric Oxide 2002;6:283–94.
39 Vernet D, Ferrini MG, Valente E, Magee TR, Bou-Gharios G, Rajfer J, Gonzalez-Cadavid NF. Effect of nitric oxide on the differentiation of fibroblasts into myofibroblasts in the Peyronie's fibrotic plaque and in its rat model. Nitric Oxide 2002;7:262–76.
40 Davila H, Magee TR, Rajfer J, Gonzalez-Cadavid NF. Peyronie's disease is associated with an increase of plasminogen activator inhibitor-1 (PAI-1) at the RNA and protein levels. Urology 2005;65:645–8.
41 Gonzalez-Cadavid NF, Rajfer J. Molecular and cellular aspects of the pathophysiology of Peyronie's disease. Drug Discov Today Dis Mech 2004;1:99–106.
42 Davila HH, Magee TR, Rajfer J, Gonzalez-Cadavid NF. Gene therapy with the inducible nitric oxide synthase (iNOS) cDNA regresses the fibrotic plaque in an animal model of Peyronie's disease. Biol Reprod 2004;71:1568–77.
43 Vernet D, Qian A, Nolazco G, Cantini L, Magee TR, Ferrini MG, Rajfer J, Gonzalez-Cadavid NF. Evidence that osteogenic progenitor cells in the human tunica albuginea may originate from stem cells. Implications for Peyronie's disease. Biol Reprod 2005;73:1199–210.
44 Magee TR, Qian A, Rajfer J, Levine L, Gonzalez-Cadavid NF. Gene expression profiles in the Peyronie's disease plaque. Urology 2002;59:451–7.
45 Qian A, Meals R, Rajfer J, Gonzalez-Cadavid NF. Comparison of gene expression profiles between Peyronie's disease and Dupuytren's contracture. Urology 2004;64:399–404.
46 Valente EG, Vernet D, Ferrini MG, Qian A, Rajfer J, Gonzalez-Cadavid NF. L-arginine and PDE inhibitors counteract fibrosis in Peyronie's plaque and related fibroblast cultures. Nitric Oxide 2003;9:229–44.
47 Ferrini MG, Kovanecz I, Nolazco G, Rajfer J, Gonzalez-Cadavid NF. Effects of long-term treatment with vardenafil on the development of the fibrotic plaque in a rat model of Peyronie's disease. BJU Int 2006;97:625–33.
48 Vernet D, Magee TR, Qian A, Rajfer J, Gonzalez-Cadavid NF. Long-term continuous incubation with high doses of tadalafil does not up-regulate the levels of phosphodiesterase 5 (PDE5) in cultures of human penile smooth muscle cells. J Sex Med 2006; 3:84–94, discussion 94–5.
49 Cantini LP, Ferrini MG, Vernet D, Magee TR, Quian A, Gelfand RA, Rajfer J, Gonzalez-Cadavid NF. Pro-fibrotic role of myostatin in Peyronie's disease. J Sex Med 2008;5:1607–22.
50 Ferrini MG, Nolazco G, Vernet D, Gonzalez-Cadavid NF, Berman J. Increased vaginal oxidative stress, apoptosis, and inducible nitric oxide synthase in a diabetic rat model: Implications for vaginal fibrosis. Fertil Steril 2006;86:1152–63.
51 Gonzalez-Cadavid NF, Rajfer J. The pleiotropic effects of inducible nitric oxide synthase on the physiology and pathology of penile erection. Curr Pharm Des 2005;11:4041–6.
52 Ferrini M, Sanchez S, Vernet D, Kovanecz I, Rajfer J, Gonzalez-Cadavid NF. Age-independent penile fibrosis in the inducible nitric oxide synthase knockout (iNOS ko) mouse supports the antifibrotic role of iNOS. Soc Sex Medcin Nort Am Meeting, New York, NY, 2006.
53 Ferrini MG, Vernet D, Rivera S, Rajfer J, Gonzalez-Cadavid NF. Genetic blockade of iNOS intensifies fibrosis of the penile corpora cavernosa associated with type 1 diabetes in the iNOS knock out mouse. Abstract to be submitted to the next AUA meeting, Chicago, 2008.
54 Mulhall JP, Müller A, Donohue JF, Mullerad M, Kobylarz K, Paduch DA, Tal R, Li PS, Cohen-Gould L, Scardino PT. The functional and structural consequences of cavernous nerve injury are ameliorated by sildenafil citrate. J Sex Med 2008; 5:1126–36.
55 Aziz MT, Mostafa T, Atta H, Rashed L, Marzouk SA, Obaia EM, Sabry D, Hassouna AA, El Shehaby AM, Aziz AT. The role of PDE5 inhibitors in heme oxygenase-cGMP relationship in rat cavernous tissues. J Sex Med 2008;5:1636–45.
56 Fandel TM, Bella AJ, Lin G, Tantiwongse K, Lin CS, Pohl J, Lue TF. Intracavernous growth differentiation factor-5 therapy enhances the recovery of erectile function in a rat model of cavernous nerve injury. J Sex Med 2008;5:1866–75.

57 Mulhall JP, Müller A, Donohue JF, Golijanin D, Tal R, Akin-Olugbade Y, Kobylarz K, Cohen-Gould L, Bennett NE, Scardino P. FK506 and erectile function preservation in the cavernous nerve injury model: Optimal dosing and timing. J Sex Med 2008; 5:1334–44.
58 Pu XY, Wang XH, Gao WC, Yang ZH, Li SL, Wang HP, Wu YL. Insulin-like growth factor-1 restores erectile function in aged rats: Modulation the integrity of smooth muscle and nitric oxide-cyclic guanosine monophosphate signaling activity. J Sex Med 2008;5:1345–54.
59 Song YS, Lee HJ, Park IH, Kim WK, Ku JH, Kim SU. Potential differentiation of human mesenchymal stem cell transplanted in rat corpus cavernosum toward endothelial or smooth muscle cells. Int J Impot Res 2007;19:378–85.
60 Bivalacqua TJ, Deng W, Kendirci M, Usta MF, Robinson C, Taylor BK, Murthy SN, Champion HC, Hellstrom WJ, Kadowitz PJ. Mesenchymal stem cells alone or ex vivo gene modified with endothelial nitric oxide synthase reverse age-associated erectile dysfunction. Am J Physiol Heart Circ Physiol 2007;292:H1278–90.
61 Lin G, Hayashi N, Carrion R, Chang LJ, Lue TF, Lin CS. Improving erectile function by silencing phosphodiesterase-5. J Urol 2005;174:1142–8.
62 Artaza JN, Singh R, Ferrini MG, Braga M, Tsao J, Gonzalez-Cadavid NF. Myostatin promotes a fibrotic phenotypic switch in multipotent C3h 10T1/2 cells without affecting their differentiation into myofibroblasts. J Endocrinol 2008;196:235–49.
63 Magee TR, Artaza JN, Ferrini MG, Zuniga FI, Cantini L, Reisz-Porszasz S, Rajfer J, Gonzalez-Cadavid NF. Myostatin shRNA gene therapy increases muscle mass. J Gene Med 2006;8:1171–81.
64 Zhang LW, Piao S, Choi MJ, Shin HY, Jin HR, Kim WJ, Song SU, Han JY, Park SH, Mamura M, Kim SJ, Ryu JK, Suh JK. Role of increased penile expression of transforming growth factor-beta1 and activation of the smad signaling pathway in erectile dysfunction in streptozotocin-induced diabetic rats. J Sex Med 2008 [Epub ahead of print].
65 Atkinson JJ, Senior RM. Matrix metalloproteinase-9 in lung remodeling. Am J Respir Cell Mol Biol 2003;28:12–24.
66 Ho MH, Heydarkhan S, Vernet D, Kovanecz I, Ferrini MG, Bhatia NN, Gonzalez-Cadavid NF. Skeletal muscle-derived stem cells (MDSC) seeded on small intestinal submucosal (SIS) scaffolds stimulate vaginal repair in the rat. J Urol 2008;179:46B. (A1369).
67 Lowry WE, Richter L, Yachechko R, Pyle AD, Tchieu J, Sridharan R, Clark AT, Plath K. Generation of human induced pluripotent stem cells from dermal fibroblasts. Proc Natl Acad Sci U S A 2008;105:2883–8.
68 Ratajczak MZ, Zuba-Surma EK, Shin DM, Ratajczak J, Kucia M. Very small embryonic-like (VSEL) stem cells in adult organs and their potential role in rejuvenation of tissues and longevity. Exp Gerontol 2008 [Epub ahead of print].

# Experimental Models of Peyronie's Disease. Implications for New Therapies

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**ABSTRACT**

***Introduction.*** Despite its high prevalence and impact on the quality of life of patients, and that it is an excellent model for the study of fibrotic processes, Peyronie's disease (PD) is an orphan disease in biomedical research. The development of animal and cell culture models has advanced substantially the understanding of its molecular and cellular pathology and the proposal of new therapies.
***Aim.*** To review the literature pertaining to the use of these models for the study of PD.
***Methods.*** PubMed search conducted from the first report of an animal model for PD.
***Results.*** This model, based on the finding that transforming growth factor β1 (TGFβ1) is overexpressed in the PD plaque, consists on the injection of TGFβ1 into the tunica albuginea of the rat. This leads to a PD-like plaque retaining many of the histological and biochemical features of human PD. Another rat model, based on the hypothesis that the PD plaque arises from trauma to the penis, causing fibrinogen extravasation that initiates as fibrin a fibrotic response, consists on injection of fibrin into the tunica. The cell culture model is based on the demonstration that myofibroblasts are abundant in the human PD plaque.
***Conclusions.*** These models have: (i) clarified the role of microtrauma, myofibroblasts, and oxidative stress in plaque development; (ii) demonstrated that this tissue is under sustained turnover by fibrotic and antifibrotic mechanisms; (iii) showed the interplay of collagenolytic and fibrinolytic systems and their inhibitors; (iv) detected an endogenous antifibrotic process consisting of the expression of inducible nitric oxide synthase that counteracts oxidative stress, collagen synthesis, and myofibroblast generation; (v) characterized the antifibrotic effects of chronic treatment with phosphodiesterase type 5 (PDE5) inhibitors; (vi) discovered the cytogenetic instability of PD cells and alterations in their gene expression; and (vii) detected stem cells in the tunica albuginea with a potential role in fibrosis and ossification. **Gonzalez-Cadavid NF, and Rajfer J. Experimental models of peyronie's disease. Implications for new therapies. J Sex Med 2009;6:303–313.**

***Key Words.*** Fibrosis; TGFβ; Penis; Myofibroblast; PDE5; Myostatin

## Introduction

Except to urologists, Peyronie's disease (PD) [1–3] is a relatively unknown condition, although recent studies suggest that it may occur in up to 9% of the male population [4,5]. Because of its impact on male sexual health, PD can seriously affect the quality of life of patients and their partners [6]. Although it was described nearly 250 years ago, there still is no satisfactory medical treatment for PD. This is primarily because comparatively few research efforts have been focused

on the cellular and molecular pathology of this disorder, which is the prerequisite for finding potential therapeutic targets.

The fibrotic lesion or plaque of PD that develops in the tunica albuginea of the penis, causing penile curvature and very often pain and erectile dysfunction, recapitulates the sequence of events that characterize the development of tissue fibrosis in general. These are essentially an initial tissue insult (trauma, microtrauma, or local toxicity), followed by acute and then chronic inflammation that leads to deposition of excessive collagen and other extracellular matrix, fragmentation of elastin, and persistence of myofibroblasts. Fibrosis may then progress to partial ectopic calcification or ossification, a condition that is also seen in about 15% of PD patients [3]. In fact, before the advent of animal models for PD, and based purely on observations in human penile tissue, it was proposed that the PD plaque results from some form of trauma or microtrauma to the tunica albuginea, which allows for the accumulation of fibrin into the interstices of the tunica albuginea, ultimately leading to an abnormal wound healing process and formation of a scar [7,8]. Another seminal experimental finding was the report of increased levels of the profibrotic factor, transforming growth factor β1 (*TGFβ1*), measured within the PD plaque [9], an observation that led to the first experimental animal model for this disease.

The TGFB1 model was first proposed in 1997 by the University of California, San Francisco (UCSF) group led by Tom Lue, where they injected a TGFβ1-like peptide into the rat penile tunica albuginea [10] to produce a PD-like lesion. The second animal model for PD was described in 2003, when Davila et al. from University of California, Los Angeles (UCLA) reported the induction of a PD-like plaque similar to that of the UCSF group just by injecting fibrin itself into the tunica albuginea of the rat [11]. In addition, cell cultures from both the human PD plaque and normal tunica albuginea of the penis, originally described in 1982 by Somers et al. [12], were revisited and further characterized in 2000 by Mulhall et al. and have been proposed as an in vitro model to test some features of PD cells [13]. Taken together, these and subsequent studies on the animal and cell models performed within the last decade have helped to provide some key findings and insights into the etiology and molecular and cellular pathology of PD, and have led to the study of some potential medical treatments based on novel mechanisms as discussed below.

The clinical significance of PD and the substantial advancements in the understanding of this disease, including the proposed novel therapies facilitated by these in vitro and in vivo models, have not as yet spurred substantial funding for basic translational research on these topics. However, it is hoped that this will soon change, primarily because it would be difficult to ignore the fact that PD turns out to be an excellent model to study the processes involved in tissue fibrosis. Considering how many human disorders ultimately involve some aspect of fibrosis, it is only a matter of time before scientists in other fields discover the importance of these models, not just for PD, but for fibrosis in general.

In the current article, we have reviewed studies that focused on the development and application of these experimental animal and cell culture models that were selected in PubMed under the key words "Peyronie's," "Peyronie," and even "La Peyronie," and were published over the last decade.

## Experimental Models

### *Development and Early Characterization of the TGFβ1 Rat Model*

Based on the fact that TGFβ1 is one of the main profibrotic factors in multiple tissues [14] and the observation of an increase in TGFβ1 expression in the PD plaque [9], Lue et al. injected various concentrations of a synthetic heptopeptide, named "cytomodulin," claimed as having TGFβ1 activity, into the tunica albuginea of rats. Animals were then sacrificed at 3 days, 2 weeks, and 6 weeks [10] following the cytomodulin injection. Histochemistry using Hart and trichrome stains revealed, in comparison with saline-injected tissues, that at 6 weeks, there was substantial chronic cellular infiltration, focal and diffuse elastosis, thickening of the tunica albuginea, disorganization and clumping of collagen bundles, and expression of TGFβ1, but not of the β2 and β3 isoforms, as determined by western blot. All concentrations of cytomodulin induced TGFβ1 mRNA expression after 2 weeks. These histological features seen in the rat tunica albuginea following the cytomodulin injection are characteristic of what is seen in the human PD plaque.

The problem for other investigators to duplicate or expand on these findings involving cyto-

modulin revolved around the fact that its amino acid sequence was not published [15]. However, a subsequent article from this same group showed that the recombinant full-length TGFβ1 protein produced similar effects on the tunica as the cytomodulin. In addition, the PD-like plaque induced by the TGFβ1 protein was reduced by colchicine, a drug claimed to be able to induce collagenase and to reduce myofibroblasts. This drug has been employed clinically for the early phase of PD [16]. The efficacy of a single injection of TGFβ1 to induce a chronic inflammation and fibrosis in the tunica is probably due to the fact that this factor is able to promote its own synthesis by feedback transcriptional stimulation. Very interestingly, surgical injury of the tunica albuginea, which should mimic microtrauma in men, could only induce histological changes similar to the acute phase of PD, but not to its chronic development, and the early up-regulation of TGFβ1 protein expression was transient [17].

The TGFβ1-induced model of PD was utilized by two other groups to clarify important aspects of the etiology and pathology of the fibrotic PD plaque. First of all, in 2000, the Tulane University group showed that at 6 weeks following TGFβ1 injection into the tunica albuginea of the rat, inducible nitric oxide synthase (*iNOS*) was induced to high levels of expression while constitutive NOS was decreased, as measured by western blots in the penile tissue homogenates [18]. In addition, penile erection induced either by electrical field stimulation of the cavernosal nerve (*EFS*) or by acetylcholine was reduced following formation of a PD-like plaque in the tunica. Since aminoguanidine, an iNOS inhibitor, enhanced the erectile response to EFS, it was concluded that iNOS induction occurring in this experimental setting was deleterious to corporal smooth muscle relaxation. However, it should be reminded that nitric oxide from iNOS, if acting at all on the erectile response, may possibly stimulate it [19], and that aminoguanidine can also inhibit other biological processes, such as the formation of advanced glycosylation end products [20,21], which contribute to fibrosis by creating collagen crosslinks. These caveats complicate the interpretation of the effects of iNOS expression on corporal tissue, and on the other hand do not clarify its role in the development of fibrosis in the tunica albuginea.

Subsequently, the same group supported their early study by showing that eNOS protein and gene expression were downregulated in the corpora cavernosa of the rats that developed a TGFβ1-induced plaque in the tunica albuginea, and that nitrotyrosine, an indicator of peroxynitrite formation, was considerably increased [22]. Peroxynitrite results from the reaction of nitric oxide with reactive oxygen species (*ROS*) formed during oxidative stress, and is pro-apoptotic, so that the inference continued to be that iNOS was deleterious to the corpora cavernosa. In fact, the authors showed that arginase II, the enzyme that cleaves L-arginine and therefore reduces the NOS substrate necessary to make nitric oxide, was considerably increased by the TGFβ1 injection into the tunica albuginea, and claimed that arginase II was also induced by iNOS.

### *Characterization of the Role of the Nitric Oxide/Cyclic Guanosine Monophosphate (cGMP)/ROS Balance in the TGFβ1 Rat Model of PD and Applications of This Model*

An alternative view on the role of iNOS spontaneous induction in the TGFβ1-elicited PD-like plaque in the rat was proposed in 2002 by Ferrini et al. from UCLA after the authors noticed increased fibrosis and plaque formation in the penis when a specific inhibitor of iNOS, L-N-(1 iminoethyl) lysine acetate (L-NIL), was given to the animals for six weeks following the TGFβ1 injection into the tunica albuginea. From this observation, they proposed that iNOS operates as an antifibrotic factor in this setting [23]. The hypothesis that iNOS is antifibrotic is consistent with what has been observed in other tissues, such as the heart, liver and kidney, when fibrosis develops after these tissues have been exposed to both long-term and continuous inhibition of total nitric oxide production by a general NOS inhibitor such as Nomega-nitro-L-arginine methyl ester (L-NAME).

The first assumption of Ferrini's article was that nitric oxide was able to bind to ROS, the profibrotic compounds produced by oxidative stress, in a reaction that produces peroxynitrite. This is an apoptotic but presumably nonfibrotic compound. Nitric oxide produced from NOS isoforms would actually quench ROS, which, if left undisturbed, would elicit and maintain PD plaque development. The second assumption was that this nitric oxide did not originate from nNOS or eNOS, which are localized in the corpora cavernosa nerves and endothelium and are modulated at the enzyme

activity level by neurotransmission or hemodynamic processes. The source of this nitric oxide would be the iNOS induced within the tunical fibroblasts via transcriptional activation by cytokines. In such a setting, iNOS can produce steady levels of nitric oxide, which can then act as an endogenous defense mechanism against fibrosis. This article showed that in the human PD plaque, as compared with normal tunica, iNOS mRNA and protein were both induced. This induction occurred in parallel to collagen deposition that was estimated histochemically and by hydroxyproline levels. Both oxidative stress, as measured by hemoxygenase-I, and nitric oxide/ROS reaction, as measured by nitrotyrosinilation of proteins, accompanied these processes.

To test the role of iNOS induction in this experimental setting, the specific inhibitor of iNOS enzyme activity, L-NIL, was given in the drinking water continuously for 45 days to rats injected into the tunica with either TGFβ1 to produce the plaque or with saline as a control. It was determined that collagen deposition and oxidative stress were dramatically increased while peroxynitrite was reduced in the TGFβ1-injected tunica in comparison with saline-injected controls. This iNOS induction in the human plaque as well as the results of the L-NIL experiments suggested that iNOS was acting as a defense mechanism against fibrosis.

Further proof of the effect of iNOS was obtained when in both the human PD plaque and the TGFβ1 PD-like plaque in the rat model, myofibroblast formation was shown to have considerably increased. This was denoted by the cells expressing α-smooth muscle actin (*ASMA*), a marker of both myofibroblasts and smooth muscle cells, as compared with the ones expressing vimentin, solely a fibroblast marker [24]. Myofibrobast formation occurred in tandem with an increase in collagen synthesis, as detected by the activation of the collagen Iα gene promoter in a recombinant DNA construct. This promoter directs the expression of β-galactosidase as a reporter protein, which is very easy to detect histochemically, and was injected into the tunica albuginea of the saline or TGFβ1-injected rats 1 week before sacrifice. As expected, chronic administration of L-NIL intensified collagen synthesis, which paralleled the amount of ASMA expression.

Moreover, cell culture experiments based on the incubation of fibroblast cultures from the human PD plaque and the normal tunica albuginea using L-NIL, and cGMP and nitric oxide donors, confirmed these results. Although myofibroblasts were previously detected in the human PD plaque [13], this was the first demonstration in vivo and in vitro, and in the human PD and PD-like plaque in the rat, that myofibroblasts differentiated from normal tunica albuginea fibroblasts and increased during plaque formation. Moreover, this showed that both cGMP and nitric oxide counteracted myofibroblast accumulation. Myofibroblasts are key cells during wound healing, which, at the completion of this process, are normally eliminated by apoptosis. When they persist, this is abnormal, and such persistence leads to scar formation [25]. Moreover, their accumulation in normal tissues is, together with collagen deposition and oxidative stress, a landmark of the development of fibrosis.

These observations led to the natural conclusion that a sustained pharmacological increase of cGMP and/or nitric oxide by a long-term continuous administration of drugs such as the PDE5 inhibitors and/or nitric oxide generators, should reduce the fibrotic plaque in the TGFβ1 rat model of PD. Two subsequent articles from the UCLA group supported such a hypothesis. In the first one [26], sildenafil (as a cGMP-dependent PDE5 inhibitor), L-arginine (as a NOS substrate), and pentoxifylline (as a nonspecific cAMP-dependent PDE inhibitor) were given separately to three groups of animals in the drinking water for the 45 days following the induction of a plaque in the tunica albuginea by TGFβ1. All three compounds prevented the appearance of the PD-like plaque when assessed by collagen deposition, myofibroblast formation, and apoptosis of myofibroblasts. This was confirmed in vitro in the cell cultures from the human tunica albuginea and PD plaque, in parallel to the expected increase of cGMP or cAMP levels. Moreover, phosphodiesterase type 5 (sildenafil target) and PDE4 (pentoxifylline target) were found to be expressed in both the human and rat tunical and PD tissues and in their respective cell cultures. To our knowledge, this was the first experimental demonstration of an antifibrotic effect for PDE5 inhibitors.

Additional similar studies were performed with another PDE5 inhibitor, vardenafil, where the drug was given not only in a continuous long-term way in the drinking water as was done with sildenafil, but also in a more natural way by daily single retrolingual administration [27]. With this daily

form of administration, the plaque was similarly inhibited. Moreover, in this study, it was shown for the first time that partial regression of an already formed plaque could occur when vardenafil is given in high doses for 14 consecutive days, beginning once the plaque has already formed.

Besides these two aforementioned PD studies, our group has provided additional experimental evidence to support the view that PDE5 inhibitors administered on a long-term treatment do indeed posses antifibrotic properties. One study involved the demonstration that the normal aging related fibrotic changes in the corporal tissue can be ameliorated by these PDE5 inhibitors, while the second series of experiments involved the reduction of fibrosis of the corpora that follows cavernosal nerve injury [28]. In this latter model of cavernosal fibrosis, PDE5 inhibitors prevented the onset of cavernosal fibrosis following cavernosal nerve injury [29–31].

The TGFβ1-induced rat PD model also served to provide the initial insight into the role of another member of the TGFβ family, myostatin, which was identified in the human PD plaque [32]. Myostatin or GDF-8 (growth and differentiation factor 8) is a negative regulator of skeletal muscle mass in the human and other animals [33]. It modulates the entry of multipotent stem cells into myogenesis and adipogenesis [34], and it stimulates the development of the fibrotic phenotype of myofibroblasts from these multipotent cells [35]. The profibrotic effects of myostatin were confirmed by showing that a cDNA expressing this protein induced per se a PD plaque in the tunica albuginea [32] of the rodent, similar to that seen with TGFβ1. Although both myostatin and TGFB1 signal through a common Smad pathway, they appear to act independently in causing tunical fibrosis in the rat model of PD. This suggests that therapeutic inhibition of the Smad pathway, or common binding of both myostatin and TGFβ1 by agents like decorin, may be an effective way of controlling the progression of PD.

A recent improvement of the TGFβ1 rat model of PD appears to have reduced two of its major shortcomings, i.e., the absence of penile curvature, one of the main features of PD in men, as well as a calcification/ossification process that is seen in 15–25% of PD patients [36]. This was based on the administration of multiple injections (at 0, 3, and 6 days) of a "low" dose ($10^{10}$ particles) of a replication-deficient adenoviral construct expressing the full-length TGFβ1 protein, which, rather than just a standard single TGFβ1 injection, or a single injection of the TGFβ1 construct at higher doses, or a single injection of the recombinant TGFβ1 protein, caused a modest but evident 20-degree bending of the rat penis at 6 weeks, and no cartilage formation [37]. Unfortunately, no specific markers of ossification, such as alkaline phosphatase or von Kossa, were studied by these investigators. A similar construct expressing reporter protein β-galactosidase instead of TGFβ1 did not cause any tunical plaque, curvature, or cartilage/bone formation. Moreover, the plaque trapped inflammatory cells in the tunica and the loss of elastin fibers, although the diagnostic marker TGFβ1 was not measured, and the curvature (but not the plaque) disappeared spontaneously at 60 days. Interestingly, in this model, the plaque that was sufficient to induce penile curvature did not, however, lead to the erectile dysfunction, which had been claimed in the earlier work with TGFβ1 protein even in the absence of any curvature [18].

### *The Fibrin-Induced Model of PD and the Microtrauma Hypothesis*

As mentioned above, early articles had proposed that PD was caused by an abnormal wound healing following some form of trauma to the erect penis, thus equating the plaque with scar tissue [7,8]. It was assumed that blood proteins, among them fibrinogen, would be released from the injury into the fractured tunica albuginea and then be converted into fibrin. Persistence of this fibrin by an abnormal inhibition of the fibrinolytic system would trigger initially an acute, and later, a chronic inflammatory response, with the subsequent production of TGFβ1, reactive oxygen species, and other profibrotic factors that would then induce the development of the fibrotic plaque that characterizes PD.

In an attempt to mimic this process in an animal model, Davila et al. injected a special fibrin preparation into the rat tunica albuginea and compared this group with animals that were injected with TGFβ1 alone, with saline used as a control group, and then a group injected with both fibrin and TGFβ1 [11]. A fibrotic plaque was induced by the fibrin injection as early as 3 weeks (half of the time required for plaque formation with TGFβ1 injec-

tion). Fibrosis in this fibrin model was accompanied by the hallmarks of the TGFβ1-induced plaque, namely TGFβ1 expression, oxidative stress, myofibroblast formation, iNOS induction, peroxynitrite formation and, induction of plasminogen activator inhibitor 1 (*PAI-1*), a strong indicator that inhibition of fibrinolysis was occurring. Indeed, when the human PD plaque was then analyzed for the presence of fibrin and PAI-1, both were identified in the human tissue, thereby lending credence to the theory that the fibrin-induced PD-like lesion in the rat tunica albuginea was a potential model for the sequence of events occurring in the development of human PD.

Confirming the assumption that iNOS acts in this fibrin model as an antifibrotic agent in the same fashion as it is postulated to function in the TGFβ1 model, gene therapy with a plasmid construct of the iNOS cDNA injected in the tunica albuginea of rats induced regression of the PD-like plaque that was induced with fibrin [38]. In addition, treatment with the iNOS cDNA was also associated with a reduction in the expression of profibrotic factors and oxidative stress markers.

### *Cell Culture Models of PD and Tunica Albuginea Stem Cells*

Although the rat models have been pivotal in understanding the molecular and cellular pathology of PD, cell cultures from the human PD plaque and the normal tunica albuginea have also provided very valuable information, particularly in terms of myofibroblast differentiation, gene expression, and the role of tunical stem cells. After the initial description of myofibroblasts in the plaque and the isolation of the first cell cultures from this tissue [12], no further studies were conducted until 2000, when different cell cultures were characterized from the plaques of several PD patients and from normal human tunica albuginea [13]. The cells of the normal tunica albuginea were shown to be primarily fibroblasts. In the PD cell cultures, myofibroblasts, as well as fibroblasts, were present, and these cells demonstrated cytogenetic instability, excess production of fibrogenic cytokines, and other functional alterations [39–41]. It was proposed that PD cell cultures could be useful to devise new therapeutic strategies by trying to prevent these alterations.

In fact, PD and tunical cell cultures were used in an already cited study [24] to investigate the effect of nitric oxide and cGMP on collagen synthesis, on the presence of PDE5 and PDE4 in these cells [26], and on the up regulation of monocyte chemoattractant protein 1 (*MCP1*) in PD cells as compared with normal tunical cells [42]. MCP1 overexpression had been also characterized by DNA microarrays and confirmed by RT/PCR and western blot in human plaque tissue. These DNA microarrays also showed overexpression of other cytokines, collagen synthesis, markers of inflammation, fibroblast proliferation, and myofibroblast and osteoblast differentiation [43,44].

This was followed up by the comparison of multiple gene expression in the respective cells and tissues from both human PD and normal tunica albuginea, and in Dupuytren's nodules, a condition associated with PD [45]. The pattern of alterations of gene expression was common for certain gene families for the PD plaque tissue and cultured cells as compared with the Dupuytren's tissues, using as controls the respective normal tissues and cells. These features were essentially the upregulation of markers of myofibroblast and osteoblast differentiation as indicators of fibrosis and calcification, and paradoxically of collagen degradation (metalloproteinases 2 and 9 (MMP 2 and 9), thymosins), and of decorin, an anti-TGFβ1 factor. This would suggest that in PD tissue, there appears to be activation of an endogenous antifibrotic mechanism additional to iNOS expression, as well as the existence of active tissue turnover. The presence of tissue turnover within a scar as was seen in the PD tissue implied that the PD plaque composition was not an irreversible end point and that it may be susceptible to modulation by pharmacological intervention targeting the balance between several processes. These include at least: (i) the nitrosative reaction and oxidative stress; (ii) the TGFβ family signaling/decorin interaction; and (iii) the collagen synthesis/degradation balance. In other words, it showed that the PD plaque, as well as the Dupuytren's nodules, is in a state of flux that potentially can be pushed toward a decrease in fibrosis by stimulating or upregulating the respective endogenous defense processes.

In addition to fibrogenic cytokine expression [46,47], the PD tissue has another feature characterized in the PD cell model—namely, the fact that these cells are potentially tumorigenic or acquire this trait upon culture. This was shown by Mulhall et al. [9], who implanted these cells into SCID mice and found that subcutaneous tumors devel-

oped in all animals, whereas none occurred with fibroblast cultures from the normal tunica albuginea or foreskin [48,49]. Cultures similar to those of the human PD plaque tissue were also obtained from the rat tunica albuginea and the PD-like plaque induced by TGFβ1, but they were not tested for tumorigenesis [26].

The tumorigenic feature of PD cells was confirmed by Vernet et al. [50] by selection in soft agar, a procedure used to detect either cancer cells or hematopoietic stem cells. It was shown that these cultures express CD34 (stem cell marker), and upon incubation with TGFβ1, undergo differentiation into skeletal myofibroblasts, smooth muscle cells, and osteoblasts, but not adipocytes. In addition, these tunical cells could paracrinely modulate in dual cell culture the differentiation of a multipotent cell line into osteoblasts and myofibroblasts. The presence of stem cells in the normal tunica albuginea may explain the fibrotic and osteogenic progression of the PD plaque upon the release of cytokines following microtrauma to the penis, which would stimulate this cell lineage commitment. Since stem cells can replicate indefinitely, they may lose the control of cell replication in certain tissue environments or by a long time in culture, and thus become tumorigenic, but why this does not occur in PD remains to be determined.

Cells expressing the stem cell markers CD34 and Sca1 were also found in vivo in the normal penile tunica albuginea, as well as in the corpora cavernosa [51]. They were identified as potential endogenous stem cells, since they are likely the ones that, in the in vitro models, undergo multiple lineage differentiation, and in the PD plaque, may convert first into myofibroblasts, and, later, into osteoblasts. These stem cells may also provide a target for pharmacological therapy of PD aimed to block their role as sources for the de novo formation of the differentiated cells (myofibroblasts, osteoblasts) that cause fibrosis and ectopic ossification. In fact, the fibrotic commitment of these multipotent cells may possibly be inhibited by long-term continuous administration of PDE5 inhibitors in vivo, as discussed above. This cell culture model actually showed that a similar long-term treatment in vitro (with the PDE5 inhibitor, tadalafil) did not upregulate PDE5, thus reducing the possibility that tachyphylaxis would be an impediment for their potential daily use for long periods of time to reverse fibrosis [52].

It has recently been shown that TGFβ1 induces the Smad pathway, the common signaling for the profibrotic effects of TGFβ family members. Interferon gamma, which has been used clinically to treat PD, did not by itself abrogate the Smad pathway, but when given together with TGFβ1, the Smad signaling was stimulated. The authors concluded that interferon gamma may not be useful for PD treatment [53]. However, this interpretation must be taken with caution, since the authors did not address the possibility of interferon gamma stimulating antifibrotic pathways, like the effect that would stem from its well-known iNOS induction activity.

A recent article using tunical fibroblast cultures [54] has served to emphasize the role that TGFβ has in strongly inducing tissue inhibitors of metalloproteinases (TIMPs), well-known inhibitors of MMP, without affecting MMPs themselves, and hence in interfering with the breakdown of collagen fibers deposited in excess during the development of the PD plaque. In contrast, interleukin-1β strongly induces MMP 1, 3, 10, and 13 expression. These findings may help to explain why the overexpression of MMP 2 and 9 mRNAs previously detected in the PD plaque [45] and assumed to be an endogenous defense mechanism against fibrosis, does not translate into an intensified collagenase activity. TIMPs induced by TGFβ and other profibrotic factors may counteract this defense mechanism.

The latter article also brings to the forefront the MMP/TIMP/PAI-1 system, an interplay of collagenolytic and fibrinolytic pathways and their inhibitors, which seems to be deeply altered in PD, and the need to reexamine the potential value of specific MMP administration for the therapy of PD, or the pharmacological modulation of key factors in this interaction. The inhibition of the fibrinolytic system by high PAI-1 levels [55] may contribute to the persistence of fibrin, already shown to cause a PD-like plaque in the animal model [11], elicit TGFβ1, and increase collagen. Normal collagen content cannot then be maintained through its breakdown by MMP, because MMP activity would be reduced by high levels of TIMPs.

## Conclusions

The development of the first animal model of PD based on TGFβ1 injection into the tunica albug-

inea, and the further characterization of the PD cell cultures, initiated a decade of significant contributions to the knowledge of the molecular and cellular pathology of PD. In a way, these models had an impact on PD research similar to the one exerted by animal models of erectile dysfunction on the elucidation of the basic pathophysiological mechanisms underlying this disorder [56]. Collectively, they helped to: (i) to clarify the role of microtrauma, cytokines, myofibroblasts, and oxidative stress in plaque initiation and progression; (ii) to demonstrate that this tissue is under sustained turnover as a result of sustained fibrotic and antifibrotic mechanisms; (iii) to emphasize the interplay of the collagenolytic and fibrinolytic systems and their inhibitors in this turnover; (iv) to show that one endogenous antifibrotic process is the expression of *iNOS*, which results in nitric oxide and cGMP formation in order to inhibit oxidative stress, collagen synthesis, and myofibroblast generation; (v) to characterize the antifibrotic effects of PDE5 inhibitors given on a long term basis in order to mimic the endogenous process; (vi) the discovery of the cytogenetic instability of the PD cells and alterations in their gene expression; and (vii) the finding of stem cells in the tunica albuginea with a potential role in fibrosis and ossification.

In synthesis, the two experimental rat models share some of the histological features that were defined in the human PD plaque (e.g., [23]), such as initial inflammation and subsequent excessive deposition and disorganization of collagen fibers, elastin fragmentation, accumulation of myofibroblasts and profibrotic factors (oxidative stress, PAI-1, TGF β1, and fibrin), and of potential antifibrotic factors (iNOS), and eventual calcification and ossification. However, as it occurs with most animal models, they cannot truly represent the complexity of the human disease. Perhaps a major concern is that, sometimes, tunical fibrosis is accompanied by a similar process in the adjacent corporal tissue, although this likely results from the technical difficulty of limiting the injection exclusively to the tunical tissue. Further work may be needed in larger animals to reduce this risk. Similarly, although cell cultures from the human plaque and normal tunica contain the myofibroblasts, fibroblasts, and stem cells that in vivo participate in the fibrotic process, they cannot mimic the interplay of paracrine and juxtacrine factors that operate in vivo, including the cross-talk with corporal cells.

**Table 1** Novel pharmacological strategies for Peyronie's disease

| Basic premise: shift tissue turnover to: |
|---|
| • Predominance of collagen breakdown over synthesis |
| • Predominance of myofibroblast apoptosis over proliferation |
| • Switch off myofibroblast and osteoblast differentation from stem cells |

However, even with the limitations intrinsic to experimental vs. clinical research, these models may be improved and even used in combination to develop novel long-term pharmacological approaches to reduce the PD plaque. Just to name a few strategies, the inhibition of myofibroblast differentiation from endogenous stem cells, the stimulation of myofibroblast apoptosis, the reduction of collagen cross-linking, the induction of selective MMPs in conjunction with anti PAI-1 agents, or the combination of PDE5 inhibitors with anti-inflammatory agents (Tables 1 and 2), are potentially approachable in both the animal and cell models. They may well serve to extrapolate to PD some promising experimental studies on the molecular pathology of tissue fibrosis in general aimed to counteract this process in such organs as the liver, kidney, lung, or skin, where there is ongoing intense research activity.

Even if PD is not a life-threatening situation, the combination of its relatively high prevalence

**Table 2** Promising pharmacological targets for Peyronie's disease

| Excessive collagen deposition |
|---|
| • NO donors and PDE5 inhibitors (increase NO/cGMP, inhibit collagen synthesis). |
| • Decorin/follistatin (counteract TGF(/myostatin). |
| • Smad 7 and related (counteract downstream Smad pathway for TGF( family). |
| • β-thymosins (reduce collagen synthesis; promote healing). |
| • Pirfenidone and other antifibrotic agents (anti-inflammatory, reduce cytokines and oxidative stress). |
| • Antioxidants (reduce ROS and oxidative stress). |
| • MMP/anti-TIMP (increase collagen degradation). |
| • Endogenous MMP upregulation, activation (increase collagen degradation). |
| • Collagen cross-link breakers, Alt 711 and similar (facilitate collagen degradation) |
| Myofibroblast accumulation |
| • NO donors and PDE5 inhibitors (reduce fibroblast/stem cell differentiation, increase apoptosis). |
| • New agents targeting stem cell commitment to myofibroblast formation or syntetic phenotypic switch. |

NO = nitric oxide; PDE5 = phosphodiesterase type 5; cGMP = cyclic guanosine monophosphate; TGF(1 = transforming growth factor (1; ROS = reactive oxygen species; MMP = metalloproteinase; TIMP = tissue inhibitor of metalloproteinases.

and its impact on quality of life and public health costs, should be a call of attention for sponsoring more basic research, particularly considering the applicability of these experimental models to the elucidation of important issues in the general mechanisms of fibrotic and antifibrotic processes in a variety of diseases.


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*Conflict of Interest:* None.

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### References


1 Taylor FL, Levine LA. Peyronie's disease. Urol Clin North Am 2007;34:517–34.
2 Bella AJ, Perelman MA, Brant WO, Lue TF. Peyronie's disease (CME). J Sex Med 2007;4:1527–38.
3 Gonzalez-Cadavid NF, Rajfer J. Mechanisms of disease: New insights into the cellular and molecular pathology of Peyronie's disease. Nat Clin Pract Urol 2005;2:291–7.
4 Mulhall JP, Creech SD, Boorjian SA, Ghaly S, Kim ED, Moty A, Davis R, Hellstrom W. Subjective and objective analysis of the prevalence of Peyronie's disease in a population of men presenting for prostate cancer screening. J Urol 2004;171:2350–3.
5 Sommer F, Schwarzer U, Wassmer G, Bloch W, Braun M, Klotz T, Engelmann U. Epidemiology of Peyronie's disease. Int J Impot Res 2002;14:379–83.
6 Hellstrom WJ, Bivalacqua TJ. Peyronie's disease: Etiology, medical, and surgical therapy. J Androl 2000;21:347–54.
7 Ehrlich HP. Scar contracture: Cellular and connective tissue aspects in Peyronie's disease. J Urol 1997;157:316–9.
8 Somers KD, Dawson DM. Fibrin deposition in Peyronie's disease plaque. J Urol 1997;157:311–5.
9 El-Sakka AI, Hassoba HM, Pillarisetty RJ, Dahiya R, Lue TF. Peyronie's disease is associated with an increase in transforming growth factor-beta protein expression. J Urol 1997;158:1391–4.
10 El-Sakka AI, Hassoba HM, Chui RM, Bhatnagar RS, Dahiya R, Lue TF. An animal model of Peyronie's-like condition associated with an increase of transforming growth factor beta mRNA and protein expression. J Urol 1997;158:2284–90.
11 Davila HH, Ferrini MG, Rajfer J, Gonzalez-Cadavid NF. Fibrin as an inducer of fibrosis in the tunica albuginea of the rat: A new animal model of Peyronie's disease. BJU Int 2003;91:830–8.
12 Somers KD, Dawson DM, Wright GL Jr, Leffell MS, Rowe MJ, Bluemink GG, Vande Berg JS, Gleischman SH, Devine CJ Jr, Horton CE. Cell culture of Peyronie's disease plaque and normal penile tissue. J Urol 1982;127:585–8.
13 Anderson MS, Shankey TV, Lubrano T, Mulhall JP. Inhibition of Peyronie's plaque fibroblast proliferation by biologic agents. Int J Impot Res 2000;12(3 suppl):S25–31.
14 Wynn TA. Cellular and molecular mechanisms of fibrosis. J Pathol 2008;214:199–210.
15 El-Sakka AI, Hassan MU, Nunes L, Bhatnagar RS, Yen TS, Lue TF. Histological and ultrastructural alterations in an animal model of Peyronie's disease. Br J Urol 1998;81:445–52.
16 El-Sakka AI, Bakircioglu ME, Bhatnagar RS, Yen TS, Dahiya R, Lue TF. The effects of colchicine on a Peyronie's-like condition in an animal model. J Urol 1999;161:1980–3.

17 El-Sakka AI, Selph CA, Yen TS, Dahiya R, Lue TF. The effect of surgical trauma on rat tunica albuginea. J Urol 1998;159:1700–7.
18 Bivalacqua TJ, Diner EK, Novak TE, Vohra Y, Sikka SC, Champion HC, Kadowitz PJ, Hellstrom WJ. A rat model of Peyronie's disease associated with a decrease in erectile activity and an increase in inducible nitric oxide synthase protein expression. J Urol 2000;163:1992–8.
19 Gonzalez-Cadavid NF, Rajfer J. The pleiotropic effects of inducible nitric oxide synthase on the physiology and pathology of penile erection. Curr Pharm Des 2005;11:4041–6.
20 Jandeleit-Dahm KA, Lassila M, Allen TJ. Advanced glycation end products in diabetes associated atherosclerosis and renal disease: Interventional studies. Ann N Y Acad Sci 2005;1043: 759–66.
21 Nilsson BO. Biological effects of aminoguanidine: An update. Inflamm Res 1999;48:509–15.
22 Bivalacqua TJ, Champion HC, Leungwattanakij S, Yang DY, Hyun JS, Abdel-Mageed AB, Sikka SC, Kadowitz PJ, Hellstrom WJ. Evaluation of nitric oxide synthase and arginase in the induction of a Peyronie's-like condition in the rat. J Androl 2001;22:497–506.
23 Ferrini MG, Vernet D, Magee TR, Shahed A, Qian A, Rajfer J, Gonzalez-Cadavid NF. Antifibrotic role of inducible nitric oxide synthase. Nitric Oxide 2002;6:283–94.
24 Vernet D, Ferrini MG, Valente EG, Magee TR, Bou-Gharios G, Rajfer J, Gonzalez-Cadavid NF. Effect of nitric oxide on the differentiation of fibroblasts into myofibroblasts in the Peyronie's fibrotic plaque and in its rat model. Nitric Oxide 2002; 7:262–76.
25 Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbiani G. The myofibroblast: One function, multiple origins. Am J Pathol 2007;170:1807–16.
26 Valente EG, Vernet D, Ferrini MG, Qian A, Rajfer J, Gonzalez-Cadavid NF. L-arginine and phosphodiesterase (PDE) inhibitors counteract fibrosis in the Peyronie's fibrotic plaque and related fibroblast cultures. Nitric Oxide 2003;9:229–44.
27 Ferrini MG, Kovanecz I, Nolazco G, Rajfer J, Gonzalez-Cadavid NF. Effects of long-term vardenafil treatment on the development of fibrotic plaques in a rat model of Peyronie's disease. BJU Int 2006;97:625–33.
28 Ferrini MG, Kovanecz I, Sanchez S, Vernet D, Davila HH, Rajfer J, Gonzalez-Cadavid NF. Long-term continuous treatment with sildenafil ameliorates aging-related erectile dysfunction and the underlying corporal fibrosis. Biol Reprod 2007;76:915–23.
29 Ferrini MG, Davila H, Kovanecz I, Sanchez S, Gonzalez-Cadavid NF, Rajfer J. Long-term continuous treatment with vardenafil prevents fibrosis and preserves smooth muscle content in the rat corpora cavernosa after bilateral cavernosal nerve transaction. Urology 2006;68:429–35.
30 Kovanecz I, Rambhatla A, Ferrini MG, Vernet D, Sanchez S, Rajfer J, Gonzalez-Cadavid NF. Long term sildenafil treatment ameliorates corporal veno-occlusive dysfunction (CVOD) induced by cavernosal nerve resection in rats. Int J Impot Res 2007; 100:867–74.
31 Kovanecz I, Rambhatla A, Ferrini MG, Vernet D, Sanchez S, Rajfer J, Gonzalez-Cadavid NF. Chronic daily tadalafil prevents the corporal fibrosis and veno-occlusive dysfunction (CVOD) that occurs following cavernosal nerve resection in the rat. BJU Int 2008;101:203–10.
32 Cantini LP, Ferrini MG, Vernet D, Magee TR, Qian A, Gelfand RA, Rajfer J, Gonzalez-Cadavid NF. Profibrotic role of myostatin in Peyronie's disease. J Sex Med 2008;5:1607–22.
33 Magee TR, Artaza JN, Ferrini MG, Zuniga FI, Cantini L, Reisz-Porszasz S, Rajfer J, Gonzalez-Cadavid NF. Myostatin shRNA gene therapy increases muscle mass. J Gene Med 2006;8:1171–81.
34 Artaza JN, Bhasin S, Magee TR, Reisz-Porszasz S, Shen R, Groome NP, Meerasahib MF, Gonzalez-Cadavid NF. Myostatin inhibits myogenesis and promotes adipogenesis in C3H 10T(1/2) mesenchymal multipotent cells. Endocrinology. 2005;146: 3547–57 [Epub 2005 May 5]. Erratum in: Fareez, Meerasaluh M. Endocrinology 2006 (Oct);147:4679 [corrected to Meerasahib, Mohamed Fareez].
35 Artaza JN, Singh R, Ferrini MG, Braga M, Tsao J, Gonzalez-Cadavid NF. Myostatin promotes a fibrotic phenotypic switch in multipotent C3H 10T1/2 cells without affecting their differentiation into myofibroblasts. J Endocrinol 2008;196:235–49.
36 Hauck EW, Hackstein N, Vosshenrich R, Diemer T, Schmelz HU, Bschleipfer T, Schroeder-Printzen I, Weidner W. Diagnostic value of magnetic resonance imaging in Peyronie's disease—A comparison both with palpation and ultrasound in the evaluation of plaque formation. Eur Urol 2003;43:293–9, discussion 299–300.
37 Piao S, Ryu JK, Shin HY, Zhang L, Song SU, Han JY, Park SH, Kim JM, Kim IH, Kim SJ, Suh JK. Repeated intratunical injection of adenovirus expressing transforming growth factor-beta1 in a rat induces penile curvature with tunical fibrotic plaque: A useful model for the study of Peyronie's disease. Int J Androl 2007;31:346–53.
38 Davila HH, Magee TR, Vernet D, Rajfer J, Gonzalez-Cadavid NF. Gene transfer of inducible nitric oxide synthase complementary DNA

regresses the fibrotic plaque in an animal model of Peyronie's disease. Biol Reprod 2004;71:1568–77.
39 Mulhall JP, Nicholson B, Pierpaoli S, Lubrano T, Shankey TV. Chromosomal instability is demonstrated by fibroblasts derived from the tunica of men with Peyronie's disease. Int J Impot Res 2004; 16:288–93.
40 Mulhall JP, Branch J, Lubrano T, Shankey TV. Perturbation of cell cycle regulators in Peyronie's disease. Int J Impot Res 2001;13(5 suppl): S21–8.
41 Mulhall JP, Thom J, Lubrano T, Shankey TV. Basic fibroblast growth factor expression in Peyronie's disease. J Urol 2001;165:419–23.
42 Lin CS, Lin G, Wang Z, Maddah SA, Lue TF. Upregulation of monocyte chemoattractant protein 1 and effects of transforming growth factor-beta 1 in Peyronie's disease. Biochem Biophys Res Commun 2002;295:1014–9.
43 Magee TR, Qian A, Rajfer J, Sander FC, Levine LA, Gonzalez-Cadavid NF. Gene expression profiles in the Peyronie's disease plaque. Urology 2002;59: 451–7.
44 Gonzalez-Cadavid NF, Magee TR, Ferrini M, Qian A, Vernet D, Rajfer J. Gene expression in Peyronie's disease. Int J Impot Res 2002;14:361–74.
45 Qian A, Meals RA, Rajfer J, Gonzalez-Cadavid NF. Comparison of gene expression profiles between Peyronie's disease and Dupuytren's contracture. Urology 2004;64:399–404.
46 Mulhall JP, Branch J, Lubrano T, Shankey TV. Radiation increases fibrogenic cytokine expression by Peyronie's disease fibroblasts. J Urol 2003;170: 281–4.
47 Mulhall JP, Anderson MS, Lubrano T, Shankey TV. Peyronie's disease cell culture models: Phenotypic, genotypic and functional analyses. Int J Impot Res 2002;14:397–405.
48 Mulhall JP, Martin DJ, Lubrano T, Moser M, Kwon E, Wojcik E, Shankey TV. Peyronie's disease fibroblasts demonstrate tumorigenicity in the severe combined immunodeficient (SCID) mouse model. Int J Impot Res 2004;16:99–104.
49 Mulhall JP. Expanding the paradigm for plaque development in Peyronie's disease. Int J Impot Res 2003;15(5 suppl):S93–102.
50 Vernet D, Nolazco G, Cantini L, Magee TR, Qian A, Rajfer J, Gonzalez-Cadavid NF. Evidence that osteogenic progenitor cells in the human tunica albuginea may originate from stem cells: Implications for peyronie disease. Biol Reprod 2005;73: 1199–210.
51 Nolazco G, Kovanecz I, Vernet D, Gelfand RA, Tsao J, Ferrini MG, Magee T, Rajfer J, Gonzalez-Cadavid NF. Effect of muscle-derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. BJU Int 2008; 101:1156–64.
52 Vernet D, Magee T, Qian A, Nolazco G, Rajfer J, Gonzalez-Cadavid N. Phosphodiesterase type 5 is not upregulated by tadalafil in cultures of human penile cells. J Sex Med 2006;3:84–94, discussion 94–5.
53 Haag SM, Hauck EW, Eickelberg O, Szardening-Kirchner C, Diemer T, Weidner W. Investigation of the antifibrotic effect of IFN-gamma on fibroblasts in a cell culture model of Peyronie's disease. Eur Urol 2008;53:425–30 [Epub 2007 June 27].
54 Del Carlo M, Cole AA, Levine LA. Differential calcium independent regulation of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases by interleukin-1beta and transforming growth factor-beta in Peyronie's plaque fibroblasts. J Urol 2008;179:2447–55.
55 Davila HH, Magee TR, Zuniga FI, Rajfer J, Gonzalez-Cadavid NF. Peyronie's disease associated with increase in plasminogen activator inhibitor in fibrotic plaque. Urology 2005;65:645–8.
56 Mehta N, Sikka S, Rajasekaran M. Rat as an animal model for male erectile function evaluation in sexual medicine research. J Sex Med 2008;5:1278–83.

## BIOGRAPHICAL SKETCH

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Ferrini MG, Magee TR, Vernet D, Rajfer J, Gonzalez-Cadavid NF (2003) Penile neuronal nitric oxide synthase (PnNOS) and its regulatory proteins are present in hypothalamic and spinal cord regions involved in the control of penile erection. J Compar Neurol 458:46-61

Magee T, Zeller CB, Ferrini M, Davila H, Vernet D, Burnett AL, Rajfer J**, González-Cadavid NF** (2003) A protein inhibitor of NOS (PIN) is expressed in the rat and mouse penile nerves and co-localizes with penile neuronal NOS (PnNOS) Biol Reprod 68:478-488.

Berman JR, Almeida FG, Jolin J, Raz S, Chaudhuri G, **Gonzalez-Cadavid NF** (2003) Androgen receptors, aromatase, and 5--reductase in human vagina: correlation with menopausal status. Fertil Steril, 79:925-931.

Davila H, Ferrini M, Rajfer J, **Gonzalez-Cadavid NF** (2003) Fibrin induction of a Peyronie's-like plaque in the rat penile tunica albuginea. A new model for Peyronie's disease. Br J Urol, 91:830-838.

Reisz-Porszasz S, Bhasin S, Artaza JN, Shen R, Sinha-Hikim I, Houge A, **Gonzalez-Cadavid NF** (2003) Reduction of skeletal muscle mass in a transgenic mouse that hyperexpresses myostatin in the muscle. Am J Physiol, Am J Physiol, 2003 Oct;285(4):E876-888.

Ma K, Mallidis C, Bhasin S, Mahabadi V, Artaza J, **Gonzalez-Cadavid N**, Arias J, Salehian B (2003) Glucocorticoid-Induced Skeletal Muscle Atrophy is Associated with Upregulation of Myostatin Gene Expression. Am J Physiol, 285:E363-371.

Valente EG, Vernet D, Ferrini MG, Qian A, Rajfer J, **Gonzalez-Cadavid NF** (2003) L-arginine and PDE inhibitors counteract fibrosis in Peyronie's plaque and related fibroblast cultures. Nitric Oxide, 9:229-244.

Singh R, Artaza JN, Taylor WE, (**Gonzalez-Cadavid NF***, Bhasin S*; *: equal contributors) (2003) Androgens stimulate myogenenic differentiation and inhibit adipogenesis in C3H 10T1/2 pluripotent stem cells through an androgen receptor-mediated pathway. Endocrinology, 144:5081-508

Liu W, Thomas SG, Asa SL, **Gonzalez-Cadavid NF**, Bhasin S, Ezzat S (2003) Myostatin is a Skeletal Muscle Target of Growth Hormone Anabolic Action. J Clin Endocrin Metabol, 88:5490-5496

Bhasin S, Taylor W, Singh R, Artaza J, Sinha-hickim I, Jasuja R, Choi H, **Gonzalez-Cadavid NF** (2003) The mechanisms of androgen effects on body composition: mesenchymal pluripotent cell as the target of androgen action. J Gerontol, 58:M1103-10.

Ferrini MG, Davila H, Valente EG**, (Gonzalez-Cadavid NF***, Rajfer J*, *equal contributors) (2004) Aging-related induction of inducible nitric oxide synthase (iNOS) is vasculo-protective in the arterial media. Cardiovascular Res ;61:796-805.

Qian A, Meals R, Rajfer J, **Gonzalez-Cadavid NF** (2004) Comparison of gene expression profiles between Peyronie's disease and Dupuytren's contracture. Urology, 64:399-404.

**Gonzalez-Cadavid NF**, Bhasin S (2004) Role of myostatin in metabolism. Curr Opin Clin Nutr Metabol Care, 7:451-457.

**Gonzalez-Cadavid NF**, Rajfer J (2004). Therapy of erectile function: Potential future treatments. Endocrine, 23:167-176.

**Gonzalez-Cadavid NF,** Rajfer J (2004) Molecular and cellular aspects of the pathophysiology of Peyronie's disease. Drug Discovery Today. Disease mechanisms, 1:99-106

Davila HH, Magee TR, Rajfer J, **Gonzalez-Cadavid NF** (2004) Gene therapy with the inducible nitric oxide synthase (iNOS) cDNA regresses the fibrotic plaque in an animal model of Peyronie's disease**.** Biol Reprod, 71:1568-1577

Davila HH, Rajfer J, **Gonzalez-Cadavid NF** (2004) Corporal veno-occlusive dysfunction in the aging rat. evaluation by cavernosometry and cavernosography. Urology, 64:1261-1266.

**Gonzalez-Cadavid NF**, Rajfer J (2004) Molecular pathophysiology and gene therapy of aging-related erectile dysfunction. Exptl Gerontol, 39:1705-1712

Sinha-Hikim I, Taylor WE, **Gonzalez-Cadavid NF**, Zheng W, Bhasin S (2004) Androgen receptor in human skeletal muscle and cultured muscle satellite cells: Up-regulation by androgen treatment. J Clin Endocr Metabol 89:5245-5255.

Davila H, Magee TR, Rajfer J, **Gonzalez-Cadavid NF** (2005). Peyronie's disease is associated with an increase of plasminogen activator inhibitor-1 (PAI-1) at the RNA and protein levels. Urology, 65:645-648

Hikim AP, Vera Y, Vernet D, Castanares M, Diaz-Romero M, Ferrini M, Swerdloff RS, **Gonzalez-Cadavid NF**, Wang C (2005) Involvement of nitric oxide-mediated intrinsic pathway signaling in age-related increase in germ cell apoptosis in male brown-norway rats. J Gerontol A Biol Sci Med Sci 60:702-708.

Artaza JN, Bhasin S, Magee TR, Reisz-Porszasz S, Shen R, Groome NP, Fareez MM, **Gonzalez-Cadavid NF** (2005) Myostatin inhibits myogenesis and promotes adipogenesis in C3H 10T(1/2) mesenchymal multipotent cells. Endocrinology 146:3547-3557.

Vernet D, Qian A, Nolazco G, Cantini L, Magee TR, Ferrini MG, Rajfer J, **Gonzalez-Cadavid NF** (2005) Evidence that osteogenic progenitor cells in the human tunica albuginea may originate from stem cells. Implications for Peyronie's disease. Biol Reprod, 73:1199-1210.

Jasuja R, Ramaraj P, Mac RP, Singh AB, Storer TW, Artaza J, Miller A, Singh R, Taylor WE, Lee ML, Davidson T, Sinha-Hikim I, **Gonzalez-Cadavid N**, Bhasin S (2005) Delta-4-androstene-3,17-dione binds androgen receptor, promotes myogenesis in vitro, and increases serum testosterone levels, fat-free mass, and muscle strength in hypogonadal men. J Clin Endocrinol Metab. 90:855-863.

**Gonzalez-Cadavid NF**, Rajfer J (2005) The pleiotropic effects of inducible nitric oxide synthase on the physiology and pathology of penile erection. Curr Pharm Des 11:4041-4046

Gonzalez-Cadavid NF**, Rajfer J (2005) Mechanisms of disease: new insights into the cellular and molecular pathology of Peyronie's disease. Nature Clin Pract Urol, 2:291-298.**

Saenz de Tejada I, Angulo J, Cellek S, **Gonzalez-Cadavid N**, Heaton J, Pickard R, Simonsen U (2005) Physiology of erectile function. J Sex Med 1:254-265.

Saenz de Tejada I, Angulo J, Cellek S, **Gonzalez-Cadavid N**, Heaton J, Pickard R, Simonsen U (2005) Pathophysiology of erectile dysfunction. J Sex Med 2:26-39.

Ferrini MG, Nolazco G, Vernet D, **Gonzalez-Cadavid NF***, Berman J* (*: equal contributors) (2006) Increased vaginal oxidative stress, apoptosis, and inducible nitric oxide synthase in a diabetic rat model: implications for vaginal fibrosis. Fertil Steril, 86:1152-1163.

Ferrini MG, Kovanecz I, Nolazco G, Rajfer J, **Gonzalez-Cadavid NF** (2006) Effects of long-term treatment with vardenafil on the development of the fibrotic plaque in a rat model of Peyronie's disease BJU Int, 97:625-633

Singh R, Artaza JN, Taylor WE, Braga M, Yuan X, **Gonzalez-Cadavid NF**, Bhasin S (2006) Testosterone inhibits adipogenic differentiation in 3T3-L1 cells: nuclear translocation of androgen receptor complex with beta-catenin and T-cell

factor 4 may bypass canonical Wnt signaling to down-regulate adipogenic transcription factors. Endocrinology. 2006 Jan;147(1):141-54.
Vernet D, Magee TR, Qian A, Rajfer J, **Gonzalez-Cadavid NF** (2006) Long-term continuous incubation with high doses of tadalafil does not up-regulate the levels of phosphodiesterase 5 (pde5) in cultures of human penile smooth muscle cells. J Sex Med 3:84-94; discussion 94-95
Magee TR, Artaza JN, Ferrini MG, Zuniga FI, Cantini L, Reisz-Porszasz S, Rajfer J, **Gonzalez-Cadavid NF** (2006). Myostatin shRNA gene therapy increases muscle mass. J Gene Med 8:1171-81
Kovanecz I, Ferrini MG, Vernet D, Nolazco G, Rajfer J, **Gonzalez-Cadavid NF** (2006). Pioglitazone prevents corporal veno-occlusive dysfunction (CVOD) in a rat model of type 2 diabetes melittus. BJU Int, 98:116-124
Ferrini MG, Davila H, Kovanecz I, Sanchez S, **Gonzalez-Cadavid NF*** Rajfer J* (*: equal contributors) (2006**)** Long-term continuous treatment with vardenafil prevents fibrosis and preserves smooth muscle content in the rat corpora cavernosa after bilateral cavernosal nerve transection. Urology, 2006 Aug;68(2):429-35.
Ferrini MG, Kovanecz I, Sanchez S, Vernet D, Davila HH, Rajfer J, **Gonzalez-Cadavid NF** (2007) Long-term continuous treatment with sildenafil ameliorates aging-related erectile dysfunction and the underlying corporal fibrosis. Biol Reprod, 76:915-923
Artaza JN, Reisz-Porszasz S, Dow JS, Kloner RA, Tsao J, Bhasin S, **Gonzalez-Cadavid NF** (2007) Alterations in myostatin expression are associated with changes in cardiac left ventricular mass but not ejection fraction in the mouse. J Endocrinol, 194(1):63-76.
**Gonzalez-Cadavid NF,** Rajfer J (2007) Experimental models for the study of the cellular and molecular pathophysiology of Peyronie's disease. In: Current Clinical Urology: Peyronie's Disease, A Guide to Clinical Management, ed by L.A. Levine, Humana Press, Totowa, NJ, p 19-39
Magee TR, Kovanecz I, Davila HH, Ferrini MG, Cantini L, Vernet D, Zuniga FI, Rajfer J, **Gonzalez-Cadavid NF** (2007) Antisense and short hairpin RNA (shRNA) constructs targeting PIN (protein inhibitor of NOS) ameliorate aging-related erectile dysfunction in the rat. J Sex Medic, 4(3):633-43.
Kovanecz I, Ferrini MG, Davila HH, Rajfer J, **Gonzalez-Cadavid NF** (2007) Pioglitazone ameliorates penile corpora veno-occlusive dysfunction (CVOD) in the aged rat. BJU Int. 2007 Oct; 100(4):867-74.
Kovanecz I, Rambhatla A, Ferrini MG, Vernet D, Sanchez S, Rajfer J, **Gonzalez-Cadavid NF** (2007) Long term sildenafil treatment ameliorates corporal veno-occlusive dysfunction (CVOD) induced by cavernosal nerve resection in rats. Int J Impot Res, 2007, 100(4):867-74.
Kovanecz I, Rambhatla A, Ferrini MG, Vernet D, Sanchez S, Rajfer J, **Gonzalez-Cadavid** NF (2008) Chronic daily tadalafil prevents the corporal fibrosis and veno-occlusive dysfunction (CVOD) that occurs following cavernosal nerve resection in the rat. BJU Int 101(2):203-10.
Rambhatla A, Kovanecz I, Ferrini M, **Gonzalez-Cadavid NF**, Rajfer J (2008) Rationale for phosphodiesterase 5 inhibitor use post-radical prostatectomy: experimental and clinical review. Int J Impot Res. 20(1):30-4.
Nolazco G, Kovanecz I, Vernet D, Ferrini M, Gelfand B, Tsao J, Magee T, Rajfer J, **Gonzalez-Cadavid NF** (2008) Effect of muscle derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. BJU Int, 101(9):1156-64
Artaza JN, Singh R, Ferrini MG, Braga M, Tsao J, **Gonzalez-Cadavid NF** (2008) Myostatin promotes a fibrotic phenotypic switch in multipotent C3h 10T1/2 cells without affecting their differentiation into myofibroblasts. J Endocrinol 196:235-49
Cantini LP, Ferrini MG, Vernet D, Magee TR, Quian A, Gelfand RA, Rajfer J, **Gonzalez-Cadavid NF** (2008) Pro-fibrotic role of myostatin in Peyronie's disease. J Sex Med, 5(7):1607-22
**Gonzalez-Cadavid NF**, Rajfer J. Experimental models for Peyronie's disease. Implications for therapy. J Sex Medic, 2008, in press
Ferrini MG, Kovanecz I, Sanchez S, Vernet D, Umeh C, Rajfer J, **Gonzalez-Cadavid NF.** Fibrosis and loss of smooth muscle in the corpora cavernosa precede corporal veno-occlusive dysfunction (CVOD) induced by experimental cavernosal nerve damage in the rat. J Sex Medic, 2008, in press
Braga M, Sinha Hikim AP, Datta S, Ferrini MG, Brown D, Kovacheva EL, Gonzalez-Cadavid NF, Sinha-Hikim I (2008) Involvement of oxidative stress and caspase 2-mediated intrinsic pathway signaling in age-related increase in muscle cell apoptosis in mice. Apoptosis. 2008 Jun;13(6):822-32.
Kovanecz I, Nolazco G, Ferrini MG, Toblli, JE, Heydarkhan S, Vernet D, Rajfer J, **Gonzalez-Cadavid NF** (2008) Early onset of fibrosis within the arterial media in a rat model of type 2 diabetes mellitus exhibiting erectile dysfunction. BJU Int, 2008, in press.
Gonzalez-Cadavid NF (2008) Mechanisms of penile fibrosis. J Sex Med, in press
Toblli JE; Ferrini MG; Cao G; Vernet D; Angerosa M; **Gonzalez-Cadavid NF** (2009) Antifibrotic effects of pioglitazone on the kidney in a rat model of type 2 diabetes mellitus. Nephrol Dial Transpl, preliminary acceptance.
Ho MH, Heydarkhan S, Vernet D, Kovanecz I, Ferrini MG, Bhatia NN, **Gonzalez-Cadavid NF** (2009) Skeletal muscle-derived stem cells seeded on small intestinal submucosal scaffolds stimulate vaginal repair in the rat. Obst Gynecol, accepted

## C. ACTIVE AND COMPLETED FUNDING

1. PR064756 (PI: Gonzalez-Cadavid) 03/01/07-02/28/10 Department of Defense
Pharmacological prevention and reversion of erectile dysfunction after radical prostatectomy, by modulation of nitric oxide/cGMP pathways
The goal is to determine whether long-term treatment with PDE5 inhibitors and nitric oxide donors can prevent corporal veno-occlusive dysfunction in a rat model of erectile dysfunction after radical prostatectomy, and whether this is due to an improvement in the underlying penile corporal fibrosis and loss of smooth muscle
No overlap
2. PC061300 (PI: Gonzalez-Cadavid) 03/31/07-02/28/11 Department of Defense
Modulation of stem cell differentiation and myostatin as an approach to counteract fibrosis in dystrophic muscle regeneration after injury.
The goal is to determine whether skeletal muscle derived stem cells (MDSC) can ameliorate skeletal muscle atrophy and fibrosis in a mouse model of Duchenne's muscular dystrophy, and this is stimulated by ex vivo gene transfer of myostatin shRNA to stem cells, and/or treatment with agents that inhibit myostatin activity
No overlap
3. NIH R21DK070003 (Gonzalez-Cadavid) 10/01/07-09/30/09 NIH NIDDK
Cell-selective expression of fibrotic gene pathways
The goal is to compare the patterns of gene expression related to fibrotic phenotypes in smooth muscle and fibroblasts in the corpora cavernosa in rat models of reproductive aging and Peyronie's disease, and the relationship between stem cells, smooth muscle cells, and fibroblasts, in myofibroblast generation in fibrosis.
No overlap
4. GCRC Medical Student Program (Gonzalez-Cadavid, PI, Wang J, student) 12/01/07-05/31/09 NIH-GCRC
Nitric oxide/cGMP modulation of skeletal muscle stem cell differentiation in myocardial infarction in the rat
The goal is to compare the antifibrotic and tissue repair effects of a long-term continuous treatment with low and high doses of a PDE5 inhibitor with or without concurrent treatment with skeletal muscle derived stem cells and determine whether this pharmacological intervention modulates endogenous and exogenous stem cell differentiation into cardiomyocytes
No overlap

**Research Support (currently submitted)**

1. RO1 DK5306907 (PI: Gonzalez-Cadavid), 05/01/03-04/30/08 NIH/NIDDK. Renewal submitted: 03/05/09
Erectile Dysfunction and Nitric Oxide Synthase in Aging
The goal is to apply novel procedures of gene and stem cell therapy for the treatment of aging-related erectile dysfunction, based on the modulation of the nitric oxide/cGMP pathway in the corpora cavernosa in a rat model of reproductive aging, and whether this restores nitrergic neurotransmission and/or corporal smooth muscle
No overlap
2. R21 DK (PI: Gonzalez-Cadavid), 12/01/09-11/30/11 NIH/NIDDK. Submitted: 02/17/09
PPAR gamma modulation of Oct 4 kidney stem cells in diabetic nephropathy
The goal is to determine whether cells that express the embryonic stem cell gene Oct-4 in the adult kidney and that are visualized by green fluorescence in a transgenic mouse that expresses gfp under the Oct-4 promoter, are true stem cells that intervene in renal repair and that PPARγ agonists at doses that do not exert glycemic control, can still counteract the reduction of their number and differentiation ability caused by diabetes
No overlap

## BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| NAME | POSITION TITLE |
|---|---|
| Rajfer, Jacob | Chief of Urology, Harbor-UCLA Medical Center |
| eRA COMMONS USER NAME (credential, e.g., agency login)<br>JRAJFER | |

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | YEAR(s) | FIELD OF STUDY |
|---|---|---|---|
| University of Illinois, Chicago, Illinois | B.S. | 1968 | Biology |
| Northwestern University, Chicago, Illinois | M.D. | 1972 | Medicine |
| L.A. County-USC Med Ctr. Los Angeles, CA | Internship | 1973 | Medicine |
| St.Joseph's Hospital, Denver, Colorado | Residency | 1974 | Surgery |
| The Johns Hopkins Hospital, Baltimore, MD | Residency | 1978 | Urology |

### A. Position and Honors

**Positions and Employment**

1978-80 Chief of Urology, Veterans Administration Med. Center, Seattle, Washington
1978-80 Assistant Professor, Dept. of Urology, University of Washington, Seattle, Washington
1980-83 Assistant Professor, Division of Urology, Department of Surgery, University of California, Los Angeles, California
1983-89 Associate Professor, Division of Urology, Department of Surgery, University of California, Los Angeles, California
1989-07 Professor, Department of Urology, University of California, Los Angeles, California

1977 3rd Prize, Laboratory Research, Garyson Carrol Essay, American Urological Asso., Chicago, IL
1978 1st. Prize, Laboratory Research, Garyson Carrol Essay, American Urological Asso., Chicago, IL
1983 2nd. Prize, Joseph F. McCarthy Essay, Western Section, American Urological Asso., Vancouver, BC
1984 1st. Prize, Joseph F. McCarthy Essay, Western Section, American Urological Asso., Reno, NV
1985 1st. Prize, Joseph F. McCarthy Essay, Western Section, American Urological Asso., Anaheim, CA
1986 Honorable Mention, Research Prize Section on Urology, Am. Acad. of Pediatrics, Washington, DC
1992-07 Best Doctors in America

.

### B. Selected peer-reviewed publications

(Publications selected from 212 peer reviewed publication)


1. Krall JF, Fittingoff M, Rajfer J (1988) Characterization of cyclic nucleotide and inositol 1,4,5-triphosphate-sensitive calcium exchange activity of smooth muscle cells cultured from the human corpora cavernosa. Biol Repro 39:913-922
2. Rajfer J, Mehringer M (1990) Cavernosography following clinical failure of penile vein ligation for erectile dysfunction. J Urol 143:514-517
3. Rajfer J, Aronson EJ, Bush PA, Dorey FJ, Ignarro LJ (1992) Nitric Oxide as a mediator of relaxation of the corpus cavernosum in response to nonadrenergic, noncholinergic neurotransmission. N Eng J Med 326:90-94
4. Vernet D, Cai L, Garban H, Babbitt ML, Murray F, Rajfer J, Gonzalez-Cadavid NF (1995) Reduction of penile nitric oxide synthase in diabetic BB/WOR$^{dp}$ (Type I) and BBZ/WOR$^{dp}$ (Type II) rats with erectile dysfunction. Endocrinology, 136:5709-5717
5. Moriel EZ, Gonzalez-Cadavid NF, Ignarro LJ, Byrns R, Rajfer J (1993) Serum levels of nitric oxide metabolites do not increase during penile erection. Urology, 42:551-554

6. Magee T, Fuentes AM, Garban H, Rajavashisht T, Marquez D, Rodriguez JA, Rajfer J, Gonzalez-Cadavid NF (1996) Cloning of a novel neuronal nitric oxide synthase expressed in penis and lower urinary tract. Biochem Biophys Res Commun, 226:145-151
7. Penson D, Lugg J, Coyne C, Sadeghi F, Freedman AL, Gonzalez-Cadavid NF, Rajfer J (1997) Effect of cryptorchidism on testicular histology in a naturally cryptorchid animal model. J Urol, 158:1978-1982
8. Rendell MS, Rajfer J, Wicker PA, Smith MD for the Sidenafil Study Group Study Group. (1999) Oral sildenafil citrate (Viagra) for the treatment of erectile dysfunction in men with diabetes. JAMA. 281:421-6.
9. Gonzalez-Cadavid, Rajfer J (2002) Therapeutic stimulation of penile nitric oxide synthase (NOS) and related pathways. Drugs Today (Barc). 2000 Feb-Mar;36(2-3):163-74.
10. Magee TR, Qian A, Rajfer J, Levine L, Gonzalez-Cadavid NF (2002) Gene expression profiles in the Peyronie's disease plaque. Urology, 59:451-457
11. Ferrini MG, Vernet D, Magee TR, Shahed A, Quian A, Rajfer J, Gonzalez-Cadavid NF (2002) Antifibrotic role of inducible nitric oxide synthase (iNOS). Nitric Oxide 6:1-12
12. Magee T, Gonzalez-Cadavid NF, Rajfer J (2002). The status of gene therapy for erectile dysfunction. Contemp Urol 14:14-31
13. Ferrini MG, Magee TR, Vernet D, Rajfer J, Gonzalez-Cadavid NF (2003) Penile neuronal nitric oxide synthase (PnNOS) and its regulatory proteins are present in hypothalamic and spinal cord regions involved in the control of penile erection. J Compar Neurol 458:46-61
14. Magee T, Zeller CB, Ferrini M, Davila H, Vernet D, Burnett AL, Rajfer J, González-Cadavid NF (2003) A protein inhibitor of NOS (PIN) is expressed in the rat and mouse penile nerves and co-localizes with penile neuronal NOS (PnNOS) Biol Reprod 68:478-488.
15. Valente EG, Ferrini MG, Vernet D, Qian A, Rajfer J, Gonzalez-Cadavid NF (2003) L-arginine and PDE inhibitors counteract fibrosis in the Peyronie's fibrotic plaque and related fibroblast cultures. Nitric Oxide, 9:229-244
16. Ferrini MG, Davila H, Valente EG, Gonzalez-Cadavid NF, Rajfer J (2004) Aging-related induction of inducible nitric oxide synthase (iNOS) is vasculo-protective in the arterial media. Cardiovascular Res, 61:796-805.
17. Gore JL, Swerdloff RS and Rajfer J. (2005) Androgen deficiency in the etiology and treatment of erectile dysfunction. Urol Clin North Am 32:457-468
18. Ferrini MF, Davila HH, Kovanecz I, Sanchez S, Gonzalez-Cadavid NF and Rajfer J. (2006) Vardenafil prevents the fibrosis and loss of corporal smooth muscle following bilateral cavernosal nerve resection in the rat. Urology 68:429-435.
19. Shabsigh R, Rajfer J, Aversa A, Traish AM, Yassin A, Kalinchenko SY and Buvat J. (2006) The evolving role of testosterone in the treatment of erectile dysfunction. Int J Clin Pract 60:1087-1092.
20. Rajfer J, Aliotta PJ, Steidle CP, Fitch WP, Zhao Y and Yu A. (2007) Tadalafil dosed once a day in men with erectile dysfunction: a randomized, double-blind, placebo controlled study in the United States. Intl J Imp Res 19:95-103.
21. Ferrini MG, Kovanecz I, Sanchez S, Vernet D, Davila HH, Rajfer J and Gonzalez-Cadavid NF. (2007) Long-term continuous treatment with sildenafil ameliorates aging-related erectile dysfunction and the underlying corporal fibrosis in the rat. Biol Reprod 76:915-23.
22. Rambhatla A, Kovanecz I, Ferrini MF, Gonzalez-Cadavid NF and Rajfer J. (2008) Rationale for phosphodiesterase 5 inhibitor use post radical prostatectomy – experimental and clinical review. Int J Imp Res, 20:30-34.
23. Kovanecz I, Rambhatla A, Ferrini MG, Vernet D, Sanchez S, Rajfer J and Gonzalez-Cadavid N. (2008) Chronic daily tadalafil prevents the corporal fibrosis and veno-occlusive dysfunction that occurs after cavernosal nerve resection. BJU Int 101:203-210
24. Nolazco G, Kovanecz I, Vernet D, Ferrini M, Gelfand B, Tsao J, Magee T, Rajfer J, Gonzalez-Cadavid NF (2008) Effect of muscle derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. BJU Int, 101(9):1156-64

25. Cantini LP, Ferrini MG, Vernet D, Magee TR, Quian A, Gelfand RA, Rajfer J, Gonzalez-Cadavid NF (2008) Pro-fibrotic role of myostatin in Peyronie's disease. J Sex Med, 5(7):1607-22
26. Gonzalez-Cadavid NF, Rajfer J. Experimental models for Peyronie's disease. Implications for therapy. J Sex Medic, 2008, in press
27. Ferrini MG, Kovanecz I, Sanchez S, Vernet D, Umeh C, Rajfer J, Gonzalez-Cadavid NF. Fibrosis and loss of smooth muscle in the corpora cavernosa precede corporal veno-occlusive dysfunction (CVOD) induced by experimental cavernosal nerve damage in the rat. J Sex Medic, 2008, in press
28. Kovanecz I, Nolazco G, Ferrini MG, Toblli, JE, Heydarkhan S, Vernet D, Rajfer J, Gonzalez-Cadavid NF (2008) Early onset of fibrosis within the arterial media in a rat model of type 2 diabetes mellitus exhibiting erectile dysfunction. BJU Int, 2008, in press.

**C. Active and Completed Funding**

R01 DK53069 05/03-04/08
NIH/NIDDK
Erectile Dysfunction and Nitric Oxide Synthase in Aging
To apply novel procedures of gene and stem cell therapy for the treatment of aging related erectile dysfunction based on modulation of the NO/cGMP pathway in the corporal tissue of the rat.
Role: Co-I 3% (non compensated)

N737500/53-5128-4380 01/05-12/07
Los Angeles County/Lance Armstrong Foundation
Los Angeles County Germ Cell Tumor & Tissue Bank Resources at USC
To develop a tissue bank of testis tumors in Los Angeles County for future use by scientific investigators.
Role: PI 2% (non compensated)

R21 DK070003 07/07-06/09
NIH/NIDDK
Cell-Selective Expression of Fibrotic Gene Pathways
To compare the patterns of gene expression related to fibrosis in the smooth muscle and fibroblasts of the corpora cavernosa and tunica albuginea, respectively, of the penis and to determine the relationship between stem cells, smooth muscle cells and fibroblasts in the generation of myofibroblasts in fibrosis.
Role: Co-I 3% (non compensated)

American Diabetes Association 08/05-07/08
Erectile dysfunction and vascular fibrosis in diabetes
To evaluate the role of NO/cGMP in preventing the fibrosis of the corporal tissue and the media of the arterial wall in diabetes mellitus.
Role: Co-I 2% (non compensated)

PR 064756 03/07-02/10
Department of Defense
Pharmacological prevention and reversion of erectile dysfunction after radical prostatectomy by modulation of the NO/cGMP pathways.
To evaluate the role of NO and cGMP in preventing cellular apotosis and fibrosis in the corporal tissue following cavernosal nerve damage.
Role: Co-I 5% (non-compensated)

## BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

| NAME | POSITION TITLE |
|---|---|
| KOVANECZ, ISTVAN | Research Associate<br>Assistant Professor (appointment in progress) |
| eRA COMMONS USER NAME (credential, e.g., agency login)<br>IKOVANECZ0308 | |

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | YEAR(s) | FIELD OF STUDY |
|---|---|---|---|
| University of Szeged (former Jozsef A. University of Art and Sciences), Szeged, Hungary | M.Sc. | 1985 | Biochemistry |
| Budapest University of Technology, Institute of Continuing Engineering Education, Budapest, Hungary | CNRT | 1987 | Nuclear technology |
| University of Szeged (former Jozsef A. University of Art and Sciences), Szeged, Hungary | Ph.D | 1994 | Comparative Physiology |
| Biological Research Center of The Hungarian Academy of Sciences, Szeged, Hungary | | 1999-2000 | Genomics, IT, Bioinformatics |
| Research & Education Institute at Harbor-UCLA Medical Center, Torrance, CA, USA | | 2004 | Training course on Protected Health Information |
| LABioMed Research Institute at Harbor-UCLA Medical Center, Torrance, CA, USA | | 2004 | Training course on Protecting Study Volunteers in Research |

### A. Positions and Honors

1985-1987 Research Fellow, Institute of Genetics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary
1987-1991 Research Scientist, Blood Transfusion Center, Szent-Gyorgyi Albert Medical University, Szeged, Hungary
1991-1992 Volunteer Researcher, Department of Neurology, Mount Sinai Medical Center, CUNY, New York, NY, USA
1993-1999 Senior Research Scientist, Head of the Vivarium, Department of Pharmacology and Pharmacotherapy, Szent-Gyorgyi Albert Medical University, Szeged, Hungary
1999-2001 Biologist Chief Counselor, Institute of Genetics, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary
2000-2001 Member of the Computer Software Council of the Hungarian Academy of Sciences
2004–on Research Associate, Urology Research Laboratory, Department of Surgery, Los Angeles Biomedical Research Institute at Harbor-UCLA, Torrance, CA, USA
2008-on Assistant Professor, Dept Urology, UCLA David Geffen School Medicine, Los Angeles, CA

### B. Selected publications

#### Original research and theoretical treatises


1. Bodis-Wollner I, Antal A, **Kovanecz I**. (1993) Low-dose scopolamine and acetyl-levo-carnitine dissociate primary from cognitive visual processing in the trained monkey. *Invest Ophth Vis Sci* 34(4): 1174.
2. Antal A., **Kovanecz I,** Bodis-Wollner I. (1994) Visual discrimination and P300 are affected paralell by cholinergic agents in the behaving monkey. *Physiol Behav.* 56(1) 161-66.

3. Tagliati M., Bodis-Wollner I., **Kovanecz I,** Stanzione P. (1994) Spatial frequency tuning in the monkey retina depends on D2 receptor-linked action of dopamine. *Vision Research* 34(16):22051-57.
4. **Kovanecz I**, Csajbok E., Petri I.B. (1994) In vitro steroid sensitivity in chronic uremic and kidney transplant patients: HLA - associated susceptibility to steroid treatment. *Nephrology Dialysis Transplantation* 9(10): 1474-76.
5. **Kovanecz I**, Petri I.B., Kaiser G. (1995) HLA associated lymphocyte panel reactive (cytotoxic) antibody production in dialyzed chronic uremic patients. *Acta Microbiologica Hungarica* 42(1): 81-84.
6. **Kovanecz I,** Papp JG, Szekeres L. (1997) Increased cardiac workload by adrenoreceptor agonists for the estimation of potential antiischemic activity in a conscious rabbit model. *J Pharmacol Toxicol Methods* 37(3): 149-59.
7. Szekeres L, **Kovanecz I**, Papp JG. (1997) Delayed cardiac adaptation to stress moderates response to beta-adrenoceptor agonists. *J Mol Cell Cardiol* 29(5): A134.
8. **Kovanecz I**, Ábrahám A, Makay G, Lukács E, Szekeres L, Papp JGy. (1997) Delayed cardiac adaptation to ischaemic stress - limitation of infarct size in a rabbit model of ischaemia-reperfusion by a single dose of iloprost. 1997 . *J Mol Cell Cardiol* 29(5): A89.
9. Takase H, **Kovanecz I**, Mori T. et al. (2003) Acute and anti-ischemic actions of pranipidine in three animal models. *Asia Pacific Journal of Pharmacology* 16(1): 29-37.
10. Davila HH, Miranda-Sousa AJ, **Kovanecz I**, et al. Effect of bilateral cavernosal nerve resection on the histological alteration in the penile vascular system. *J Urol* 2005; 173(4S): 288.
11. Ferrini MG, **Kovanecz I**, Nolazco G, Rajfer J, Gonzalez-Cadavid NF. (2006) Effects of long-term vardenafil treatment on the development of fibrotic plaques in a rat model of Peyronie's disease. *BJU Int*. 97(3):625-33.
12. **Kovanecz I**, Ferrini MG, Vernet D, Nolazco G, Rajfer J, Gonzalez-Cadavid NF. (2006) Pioglitazone prevents corporal veno-occlusive dysfunction (CVOD) in a rat model of type 2 diabetes mellitus. *BJU Int*. 98:116-24
13. Ferrini MG, Davila HH, **Kovanecz I**, Sanchez SP, Gonzalez-Cadavid NF, Rajfer J. Vardenafil prevents fibrosis and loss of corporal smooth muscle that occurs after bilateral cavernosal nerve resection in the rat. *Urology* 2006; 68:429-35
14. Ferrini MG, **Kovanecz I**, Sanchez S, Vernet D, Davila HH, Rajfer JA, Gonzalez-Cadavid NF. (2007) Long-term continuous treatment with sildenafil ameliorates aging-related erectile dysfunction and the underlying corporal fibrosis in the rat. *Biol Reprod.* 76(5):915-23.
15. Magee TR, **Kovanecz I**, Davila HH, Ferrini MG, Cantini L, Vernet D, Zuniga FI, Rajfer J, Gonzalez-Cadavid NF. (2007) Antisense and short hairpin RNA (shRNA) constructs targeting PIN (Protein Iinhibitor of NOS) ameliorate aging-related erectile dysfunction in the rat. *J Sex Med* 4(3):633-43.
16. **Kovanecz I**, Ferrini MG, Vernet D, Nolazco G, Rajfer J, Gonzalez-Cadavid NF. (2007) Aging-related copora veno-occlusive dysfunction in the rat is ameliorated by pioglitazone. *BJU Int* 100(4):867-74.
17. **Kovanecz I**, Rambhatla A, Ferrini MG, Rajfer J, Gonzalez-Cadavid NF. (2007) Long term sildenafil treatment ameliorates corpora veno-occlusive dysfunction (CVOD) induced by cavernosal nerve resection in rats. *Int J Impot Res* Sep 20; [Epub ahead of print]
18. **Kovanecz I**, Rambhatla A, Ferrini MG, Rajfer J, Gonzalez-Cadavid NF. (2008) Chronic daily tadalafil prevents the corporal fibrosis and veno-occlusive dysfunction that occurs after cavernosal nerve resection. *BJU Int.* 101(2):203-10.
19. Nolazco D, **Kovanecz I**, Vernet D, Ferrini MG, Gelfand B, Tsao J, Mage T, Rajfer J, Gonzalez-Cadavid NF. (2008) Effect of muscle derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. *BJU Int* Feb 21; [Epub ahead of print]
20. **Kovanecz I**, Nolazco G, Ferrini MG, Toblli JE, Heydarkhan S, Vernet D, Rajfer J, Gonzalez-Cadavid NF. Early onset of fibrosis within the arterial media in a rat model of type 2 diabetes mellitus with erectile dysfunction. BJU Int. 2009 Jan 9. [Epub ahead of print]
21. Ferrini MG, **Kovanecz I**, Sanchez S, Umeh C, Rajfer J, Gonzalez-Cadavid NF. Fibrosis and Loss of Smooth Muscle in the Corpora Cavernosa Precede Corporal Veno-Occlusive Dysfunction (CVOD) Induced by Experimental Cavernosal Nerve Damage in the Rat. J Sex Med. 2008 Dec 2. [Epub ahead of print]

**Non-experimental articles**

1. Rambhatla A, **Kovanecz I,** Ferrini M, Gonzalez-Cadavid NF, Rajfer J. (2008) Rationale For PDE5 Inhibitor Use Post Prostatectomy. *Int J Impot Res* 20(1):30-34. [Epub 2007 Aug 2.]

**C. Active and Completed Funding.**

None

## BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

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| Vernet, Agueda Dolores | Research Associate |
| eRA COMMONS USER NAME (credential, e.g., agency login)<br>avernet0507 | |

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

| INSTITUTION AND LOCATION | DEGREE *(if applicable)* | YEAR(s) | FIELD OF STUDY |
|---|---|---|---|
| Central Univ of Venezuela, Caracas, Venezuela | Biologist | 1976 | Cellular Biology |
| Venezuelan Institute Scientific Res, Venezuela | M.Sc. | 1983 | Immunology |
| Central Univ of Venezuela, Caracas, Venezuela | Ph.D. | 1993 | Cellular Biology |

### A. Postitions and Honors

1976-83 Biologist, Institute of Legal Medicine, Caracas, Venezuela
1980-81 Visiting Scientist, University of Compiègne, Compiègne, France
1983-84 Biologist, Amazonic Center of Research of Tropical Diseases, Caracas, Venezuelas Instructor Professor, Dept Biochem, Fac Medicine, Central Univ Venezuela, Caracas, Venezuela.
1991 Research Associate, Dept Surgery, Harbor-UCLA Medical Center, Torrance, California
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### B. Publications


1. San Blas G, **D Vernet** (1975) Induction of the synthesis of the cell wall $\alpha$-1,3-glucan in the yeast-like form of Paracoccidioides brasiliensis strain IVIC Pb9 by fetal calf serum. Infect & Immun 15:897-902.
2. Gonzalez-Cadavid NF, **D Vernet,** A Fuentes, JA Rodriguez, RS Swerloff, J Rajfer (1993) Up-regulation of levels of androgen receptor and its mRNA by androgens in smooth-muscle cells from rat penis. Molec Cell Endocrinol 90:219-229
3. Garban H, **D Vernet**, A Freedman, J Rajfer, NF Gonzalez-Cadavid (1995) Effect of aging on nitric oxide-mediated penile erection in rats. Am J Physiol 268: (Heart Circ Physiol) H467-H475
4. Hung A, **D Vernet**, Y Xie, T Rajavashisth, JA Rodriguez, J Rajfer, NF Gonzalez-Cadavid (1995) Expression of the inducible nitric oxide synthase in smooth muscle cells from the rat penile corpora cavernosa. J Androl 16:469-481
5. **Vernet D**, L Cai, H Garban, ML Babbit, F Murray, J Rajfer, NF Gonzalez-Cadavid (1995) Reduction of penile nitric oxide synthase in diabetic BB/WOR$^{dp}$ (Type I) rats with erectile dysfunction. Endocrinology 136:5709-5717.
6. Garban H, D Marquez, T Magee, J Moody, T Rajavashisht, JA Rodriguez, A Hung, **D Vernet**, J Rajfer, NF Gonzalez-Cadavid (1997) Cloning of rat and human inducible nitric oxide synthase. Application for gene therapy of erectile dysfunction. Biol Reproduct 56, 954 -963.
7. Moody J, **Vernet D**, S Laidlaw, J Rajfer, NF Gonzalez-Cadavid (1997) Effects of long-term administration of L-arginine on the rat erectile response. J Urol 158, 942-947
8. **Vernet D**, Bonavera JJ, Swerloff R, Gonzalez-Cadavid NF, Wang C. (1998) Spontaneous expression of inducible nitric oxide synthase (iNOS) in the hypothalamus and other brain regions of aging rats. Endocrinology, 139(7): 3254-3261

9. González-Cadavid NF, Burnett AL, Magee T, Zeller CB, **Vernet D**, Gitter J, Smith N, Rajfer J (2000) Expression of penile neuronal nitric oxide synthase variants in the rat and mouse penile nerves. Biol Reprod, 63:704-714

10. González-Cadavid NF, Ryndin I, **Vernet D**, Magee TR, Rajfer J (2000) Presence of NMDA receptor subunits in the male lower urogenital tract. J Androl 21:566-578

11. Ferrini M, Magee TR, **Vernet D**, Rajfer J, Gonzalez-Cadavid NF (2001) Aging-related expression of inducible nitric oxide synthase (iNOS) and markers of tissue damage in the rat penis. Biol Reprod, 64:974-982

12. Wang C, Sinha Hikim A, Ferrini M, Bonavera JJ, **Vernet D**, Leung A, Lue-YH, Gonzalez-Cadavid NF, Swerdloff RS (2002) Male reproductive aging: using the Brown Norway rat as a model for man. In: "Endocrine facets of aging in the human and experimental animal" Novartis Found Symp 242:82-95

13. Ferrini MG, **Vernet D**, Magee TR, Shahed A, Quian A, Rajfer J, Gonzalez-Cadavid NF (2002) Antifibrotic role of inducible nitric oxide synthase (iNOS). Nitric Oxide 6:1-12

14. Gonzalez-Cadavid NF, Magee TR, Ferrini M, Qian A, **Vernet D**, Rajfer J (2002) Gene expression in Peyronie's disease. Int J Impot Res, 14:361-374**.**

15. Magee TR, Ferrini M, Garban H, **Vernet D**, Mitani K, Rajfer J, Gonzalez-Cadavid NF (2002) Gene therapy of erectile dysfunction in the rat with penile neuronal nitric oxide synthase cDNA. Biol Reprod, 67:1033-1041.

16. **Vernet D**, Ferrini MG, Valente E, Magee TR, Bou-Gharios G, Rajfer J, Gonzalez-Cadavid NF (2002) Effect of nitric oxide on fibroblast differentiation into myofibroblasts in cell cultures from the Peyronie's fibrotic plaque and in its rat model in vivo. Nitric Oxide 7:262-276.

17. Ferrini MG, Magee TR, **Vernet D**, Rajfer J, González-Cadavid NF (2003) Penile neuronal nitric oxide synthase (PnNOS) and its regulatory proteins are present in hypothalamic and spinal cord regions involved in the control of penile erection. J Compar Neurol 458:46-61

18. Magee TR, Ferrini MG, Davila HH, Zeller CB, **Vernet D**, Sun J, Lalani R, Burnett AL, Rajfer J, González-Cadavid NF. (2003) Protein inhibitor of nitric oxide synthase (NOS) and the N-methyl-D-aspartate receptor are expressed in the rat and mouse penile nerves and colocalize with penile neuronal NOS. Biol Reprod 68:478-488.

19. Valente EG, **Vernet D**, Ferrini MG, Qian A, Rajfer J, González-Cadavid NF (2003) L-arginine and (PDE) inhibitors counteract fibrosis in the Peyronie's fibrotic plaque and related fibroblast cultures. Nitric Oxide, 9:229-244

20 Davila HH, Magee TR, **Vernet D**, Rajfer J, González-Cadavid NF. (2004) Gene transfer of inducible nitric oxide synthase cDNA regresses the fibrotic plaque in an animal model of Peyronie's disease. Biol Reprod 71(5).90

21. Sinha Hikim AP, Vera Y, **Vernet D**, Castanares M, Diaz-Romero M, Ferrini M, Swerdloff RS, González-Cadavid NF, Wang C. (2005) Involvement of nitric oxide-mediated intrinsic pathway signaling in age-related increase in germ cell apoptosis in male brown-norway rats. J Gerontol A Biol Sci Med Sci. 60(6):702-8.

22. **Vernet D**, Qian A, Nolazco G, Cantini L, Magee TR, Ferrini MG, Rajfer J, González-Cadavid NF (2005) Evidence that osteogenic progenitor cells in the human tunica albuginea may originate from stem cells. Implications for Peyronie's disease. Biol Reprod, 73:1199-1210

23. **Vernet D**, Magee TR, Qian A, Nolazco G, Rajfer J, González-Cadavid NF. (2006) Phosphodiesterase type 5 is not upregulated by tadalafil in cultures of human penile cells. J Sexual Medicine,3:84-95.

25. Magee TR, Artaza JN, Ferrini MG, **Vernet D**, Zuniga FI, Cantini L, Reisz-Porszasz, Rajfer J, González-Cadavid, NF. (2006) Myostatin short interfering hairpin RNA gene transfer increases muscle mass. J Gene Med, 8(9):1171-81.

26. Kovanecz I, Ferrini MG, **Vernet D**, Nolazco G, Rajfer J, González-Cadavid NF (2006). Pioglitazone prevents corporal veno-occlusive dysfunction in a rat model of type 2 diabetes mellitus. British J Urology, 98(1):116-124

27. Ferrini MG, Nolazco G, **Vernet D**, González-Cadavid NF, Berman J. (2006). Increased vaginal oxidative stress, apoptosis, and inducible nitric oxide synthase in a diabetic rat model: implications for vaginal fibrosis. Fertil Steril, 86 Suppl 4:1152-63; [Epub ahead of print]

28. Ferrini MG, Kovanecz I, Sanchez S, **Vernet D**, Davila HH, Rajfer J, Gonzalez-Cadavid NF. (2007) Long-Term Continuous Treatment with Sildenafil Ameliorates Aging-Related Erectile Dysfunction and the Underlying Corporal Fibrosis in the Rat. Biol Reprod Feb 14; [Epub ahead of print]

29. Magee TR, Kovanecz I, Davila H, Ferrini M, Cantini L, **Vernet D**, Zuniga F, Rajfer J, Gonzalez-Cadavid NF. (2007) Antisense and short hairpin RNA (shRNA) constructs targeting PIN (Protein Inhibitor of NOS) ameliorate aging-related erectile dysfunction in the rat. J Sex Med, 4(3):633-43

30. Kovanecz I, Rambhatla A, Ferrini MG, **Vernet D**, Sanchez S, Rajfer J, Gonzalez-Cadavid NF (2007) Long term sildenafil treatment ameliorates corporal veno-occlusive dysfunction (CVOD) induced by cavernosal nerve resection in rats. Int J Impot Res, 2007, 100(4):867-74.

31. Kovanecz I, Rambhatla A, Ferrini MG, **Vernet D**, Sanchez S, Rajfer J, Gonzalez-Cadavid NF (2008) Chronic daily tadalafil prevents the corporal fibrosis and veno-occlusive dysfunction (CVOD) that occurs following cavernosal nerve resection in the rat. Brit J Urol Int 101(2):203-10.

32. Nolazco G, Kovanecz I, **Vernet D**, Ferrini M, Gelfand B, Tsao J, Magee T, Rajfer J, Gonzalez-Cadavid NF (2008) Effect of muscle derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. British JUrol Int, 101(9):1156-64. Epub 2008 Feb 21.

33. Cantini LP, Ferrini MG, **Vernet D**, Magee TR, Quian A, Gelfand RA, Rajfer J, Gonzalez-Cadavid NF (2008) Pro-fibrotic role of myostatin in Peyronie's disease. J Sex Med, [Epub ahead of print]

34. Ferrini MG, Kovanecz I, Sanchez S, **Vernet D**, Umeh C, Rajfer J, Gonzalez-Cadavid NF. Fibrosis and loss of smooth muscle in the corpora cavernosa precede corporal veno-occlusive dysfunction (CVOD) induced by experimental cavernosal nerve damage in the rat. J Sex Medic, 2008, in press

35. Kovanecz I, Nolazco G, Ferrini MG, Toblli, JE, Heydarkhan S, **Vernet D**, Rajfer J, Gonzalez-Cadavid NF (2008) Early onset of fibrosis within the arterial media in a rat model of type 2 diabetes mellitus exhibiting erectile dysfunction. BJU Int, 2008, in press.

36. Toblli JE; Ferrini MG; Cao G; **Vernet D**; Angerosa M; Gonzalez-Cadavid NF (2009) Antifibrotic effects of pioglitazone on the kidney in a rat model of type 2 diabetes mellitus. Nephrol Dial Transpl, preliminary acceptance.

37. Ho MH, Heydarkhan S, **Vernet D**, Kovanecz I, Ferrini MG, Bhatia NN, Gonzalez-Cadavid NF (2009) Skeletal muscle-derived stem cells seeded on small intestinal submucosal scaffolds stimulate vaginal repair in the rat. Obst Gynecol, accepted

**C. Active and Completed funding**

None

## BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

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2004-2007 Research Associate at Dept Surgery/Urology Research Laboratory Los Angeles Biomedical Research Institute at Harbor-UCLA, Torrance CA.

2007- on Research Associate at Charles Drew University School of Medicine, Los Angeles CA.

**B. Publications.**


**1. Nolazco GM,** Ramirez J. (2000) Determination of specific acetylcholine receptor subunit expression in identified neurons found in the marine mollusk, *Aplysia californica*. *Dimensions*, 2: 14-19. (Undergraduate student research publication at CSU Fullerton)

**2. Nolazco GM**. (2003) Identification and developmental expression of alternatively spliced transcripts of the small conductance calcium-activated potassium channel in *Drosophila melanogaster*, Master's Thesis, California State University Fullerton.

**3.** Vernet D, **Nolazco G**, Cantini L, Magee TR, Qian A, Rajfer J, Gonzalez-Cadavid NF. (2005) Evidence that osteogenic progenitor cells in the human tunica albuginea may originate from stem cells: implications for peyronie disease. Biol Reprod, 73(6): 1199-210.

**4.** Vernet D, Magee TR, Qian A, **Nolazco G,** Rajfer J, Gonzalez-Cadavid NF. (2006) Phosphodiesterase type 5 is not upregulated by Tadalafil in cultures of human penile cells. J Sex Medicine, (3(1): 4-95.

**5.** Ferrini MG., Kovanecz I, **Nolazco G**, Rajfer J, Gonzalez-Cadavid NF (2006) Effects of long-term vardenafil treatment on the development of fibrotic plaques in a rat model of Peyronie's disease. Br J Urol, 97 (3): 625-33.

**6.** Kovanecz I, Ferrini MG, Vernet D, **Nolazco G**, Rajfer J., Gonzalez-Cadavid NF (2006) Pioglitazone prevents corporal veno-occlusive dysfunction (CVOD) in a rat model of type 2 diabetes mellitus. Br J Urol, 98(1):6-24.

**7.** Ferrini MG, **Nolazco G**, Vernet D, Gonzalez-Cadavid NF, Berman J. (2006) Increased vaginal oxidative stress, apoptosis, and inducible nitric oxide synthase, in a diabetic rat model: implications for vaginal fibrosis. J Fertil and Steril, 86 Suppl 4: 1152-63.

**8.** Kovanecz I., Ferrini MG, Vernet D, **Nolazco G**, Rajfer J, Gonzalez-Cadavid NF (2007) Ageing-related corpora veno-occlusive dysfunction in the rat is ameliorated by pioglitazone. Br J Urol, 100 (4) 867-74.

**9. Nolazco G**, Kovanecz I, Vernet D, Gelfand RA., Tsao J, Ferrini MG., Magee TR, Rajfer J, Gonzalez-Cadavid NF. (2008) Effect of muscle derived stem cells on the restoration of corpora cavernosa smooth muscle and erectile function in the aged rat. BJU Int (9) 1156-64.

**10**. Kovanecz I, **Nolazco G**, Ferrini MG, Toblli, J, Heydarkhan S, Vernet D, Rajfer J, Gonzalez-Cadavid NF. (2008) Early onset of fibrosis within the arterial media in a rat model of type 2 diabetes mellitus exhibiting erectile disfunction BJU Int Sep 04; [Epub ahead of print]

**C. Active and Completed Funding**
None